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GENERAL SESSION
AMERICAN SOCIETY OF ANIMAL PRODUCTION
49TH ANNUAL MEETING, NOVEMBER 29, 1957
CHICAGO, ILLINOIS

INTRODUCTORY REMARKS

J. C. MILLER
President

THE industry which this organization represents has enjoyed remarkable growth and development during the past half century. We can be proud of the contributions made to American Agriculture by the membership, individually and collectively, of the American Society of Animal Production during the 49 years of its existence. The American standard of living, and our continuing increase in life expectancy are directly associated with Animal Agriculture and the high protein diet of the American people. The tremendous meat production capacity of our farms and ranches is a tribute to research and education in the broad field of Animal Science.

In spite of our accomplishments of the past, the current revolution in American Agriculture creates a real threat to the future of our industry. Can we roll with the punches of Agricultural-Industrial integration? Can we meet the competition of automation and technocracy? In short, can we compete in tomorrow's agriculture? As our past success has been largely due to research and education, so will our future be determined by the same forces.

What kind of training will be required for leadership in the field of Animal Husbandry in the future? Where will the young men come from and what curriculum retooling is necessary to provide the scientists, the farm and ranch operators and managers, and the animal husbandmen of the future? Will we train men for positions in agri-business and agri-industry as they relate to the livestock and meat business, and if so, how? Should we increase the science, mathematics and humanities and reduce the agriculture courses in our curricula? Shall we train scientists, animal husbandmen or business men, or all three? These and other similar problems are confronting colleges of agriculture throughout our land today.

In view of our declining agricultural enrollment and the need for top level men for tomorrow's agriculture, a discussion of these topics is timely. We are fortunate indeed to obtain such able men for this symposium on *Animal Husbandry Graduates in Modern Agriculture.* In the interest of time I shall introduce the speakers now, and they will follow in the order presented.

Dr. Roy M. Kottman, Associate Dean of Agriculture, Iowa State College, will discuss *"Reorientation and Modification of Objectives in Animal Husbandry Curricula."*

Mr. Herman Seinwerth, Industrial Relations Manager, Swift and Company, Chicago, will discuss *"Animal Husbandry Graduates in Industry."*

REORIENTATION AND MODIFICATION OF OBJECTIVES IN ANIMAL HUSBANDRY CURRICULA [1]

ROY M. KOTTMAN [2, 3]

THE on-rush of scientific discovery with its attendant impact on economic and social change makes it imperative that college curricula be responsive to forces of change. It is equally important that the direction of curriculum change be guided by fact rather than emotion insofar as pertinent data can be assembled.

To secure data for the study, we sent three questionnaires to each of the 48 land-grant (white) colleges and state universities. A questionnaire dealing with enrollment went to the Registrar in each institution; one dealing with job opportunities, curriculum trends, qualifications of students entering Animal Husbandry and the source of those students was sent to Heads of Animal Husbandry Departments; still a third schedule of questions was sent to Directors of Resident Instruction requesting data on curriculum changes actually accomplished, on the number of staff members employed at the present time and prior to World War II, and on average beginning salaries for the various major divisions of work represented in most of our colleges and state universities. Response to questionnaires was in all cases cordial, if not always productive of usable data. Certain items on the questionnaires were completed by as many as 37 of the 48 land-grant institutions. In other cases, as few as 6 or 7 schools were able to supply data. In most instances, however, there was sufficient response to provide data from a representative sample of schools located in each of the four regions of the United States.

One measure of public acceptance of a program is the support given it and one measure of that support is the growth made possible by that support. In Figure 1 is shown a comparison of the size of the professional staff and graduate program in Animal Husbandry for 1940–41 and the academic year 1956–57.

The data in Figure 1 indicate there has been an 87% increase in staff members of professional rank, i.e., assistant, associate and full professors. The research associate and teaching assistant numbers are several times the size that they were prior to World War II. The number of graduate assistants in the 36 institutions has increased by 840%. The total number of teachers working with undergraduates is up 58%, and the full-time equivalent teachers, i.e., the number of teachers which would be considered

[1] Prepared for a meeting of the American Society of Animal Production, Chicago, Illinois, November 29, 1957.

[2] Professor of Animal Husbandry and Associate Dean of Agriculture at Iowa State College.

[3] The author is pleased to acknowledge the assistance of Heads of Animal Husbandry Departments, Directors of Resident Instruction in Agriculture, and Registrars in the land-grant colleges and state universities from which data were obtained for this study.

on full-time teaching duty after allowance is made for a portion of their time being budgeted for research or extension, was similarly increased. Graduate students not on appointment have increased from 41 to 169, or more than fourfold in this 17-year period.

It is axiomatic that growth of the professional staff in Animal Husbandry was at least partially a response to growth in enrollment during the past 17 years. In order that we might compare increases in Animal Husbandry enrollment with that of Agriculture generally and with all-

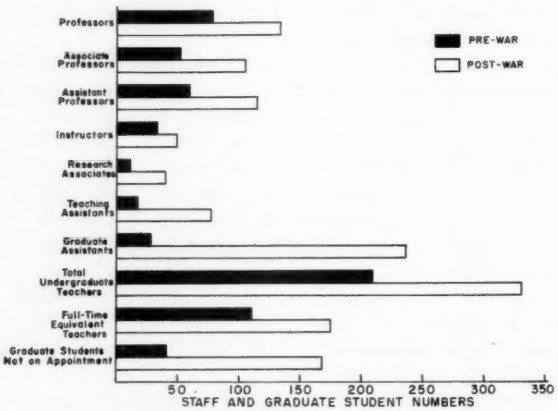

Figure 1. Size of Animal Husbandry Staff, Pre-War and Post-War.

[a] States represented: Alabama, Arizona, California, Colorado, Delaware, Florida, Idaho, Indiana, Iowa, Kansas, Louisiana, Maine, Michigan, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming.

[b] Numbers represented in the two years considered:

	1940	1956
Professors	77	134
Associate Professors	53	106
Assistant Professors	60	115
Instructors	33	49
Research Associates	11	40
Teaching Assistants	17	78
Graduate Assistants	28	236
Total Undergraduate Teachers	209	331
Full-Time Equivalent Teachers	111	176
Graduate Students Not on Appointment	41	169

college enrollment, information was sought which would give a picture of the average situation during the five-year period immediately preceding World War II, and again in the five-year period ending with the academic year 1955–56. These data are shown in Figure 2.

Data on undergraduate enrollment were received from only 12 institu-

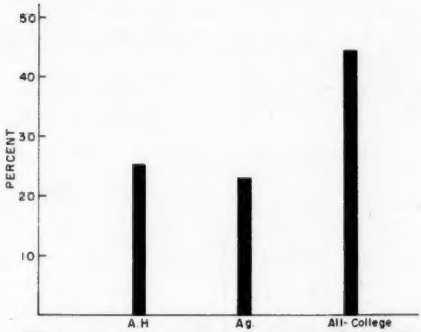

Figure 2. Increase in Post-War (1951–55) Over Pre-War (1936–40) Enrollment (Percent).

[a] States represented: Arizona, California, Colorado, Indiana, Iowa, Maine, Mississippi, Montana, Ohio, Oregon, Texas, Virginia.

[b] Percentage increase:

Animal Husbandry	26.88%
All-Agriculture	24.85
All-College	44.48

tions. However, these institutions were located in various sections of the country, hence, they are believed to be representative. It is evident from Figure 2 that Animal Husbandry has fared somewhat better than the sum-

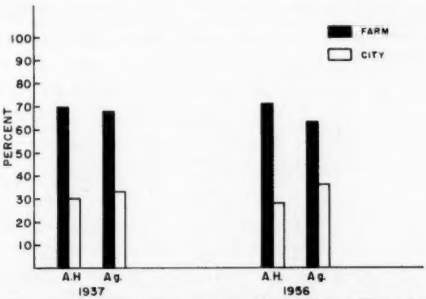

Figure 3. Pre-War and Post-War Enrollment from Farms and Urban Areas.

[a] States represented: California, Colorado, Delaware, Indiana, Iowa, New Jersey, New Mexico, North Carolina, Rhode Island, Texas, Virginia.

[b] Farm and City enrollment in 11 institutions:

		1937	1956
Animal Husbandry:	Farm	70%	71.63%
	City	30	28.37
Agriculture:	Farm	67.33	63.64
	City	32.67	36.36

total of all curricula in Agriculture, but that both Animal Husbandry and total enrollment in Agriculture have increased proportionately less than has all-college enrollment.

In considering enrollment trends, it was considered worthwhile to study the source of our students with regard to whether or not they come to us

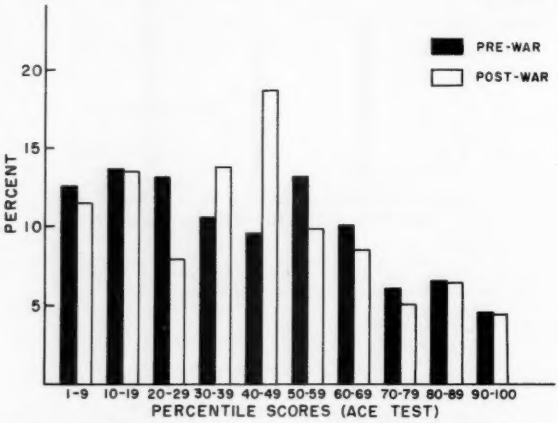

Figure 4. Proportion in Each Percentile for Freshmen in Animal Husbandry, Pre-War and Post-War.

[a] States providing data on Pre-World War II ACE scores: California, Colorado, Illinois, Iowa, Louisiana, Michigan, Washington.

[b] States providing information on 1956–57 ACE scores: Alabama, California, Colorado, Idaho, Illinois, Iowa, Louisiana, Michigan, New Hampshire, New Jersey, Oregon, Rhode Island, South Carolina, South Dakota, Texas, Virginia, Washington, West Virginia, Wyoming.

[c] Distribution of ACE scores:

	Pre-War (7 schools)		Post-War (19 schools)	
Percentiles	Number	Distribution	Number	Distribution
1–9	25	12.63%	71	11.58%
10–19	27	13.64	83	13.54
20–29	26	13.13	49	7.99
30–39	21	10.61	85	13.87
40–49	19	9.60	115	18.76
50–59	26	13.13	61	9.95
60–69	20	10.10	52	8.48
70–79	12	6.06	31	5.06
80–89	13	6.59	39	6.36
90–100	9	4.55	27	4.40

with farm backgrounds. Perhaps we should know our students today are coming from different backgrounds than those who were in our classrooms prior to World War II. Eleven institutions provided data on this point.

The data in Figure 3 came from States located in all regions, and are believed to be fairly representative of the actual situation. Surprisingly enough, about 70% of our Animal Husbandry students came from the farm in the fall of 1956, just as was the case 20 years earlier.

Enrollment from the farm has decreased from 67% to 64% for all curricula in Agriculture.

These data are possibly not representative of the situation in certain States, yet on the basis of the information presented there is little need for reorientation or modification of curricula based on pre-college residence of Animal Husbandry students.

Perhaps more important than the pre-college residence of students in Animal Husbandry is the question of mental capacity of those students. Unfortunately, only 7 schools indicated that they had available to them intelligence test records on students entering Animal Husbandry in the fall of 1937. There were, reports from 19 institutions which provided data on 1956–57 ACE scores.

One observation to be made from Figure 4 is that the situation is probably very little better or worse than it was 20 years ago with regard to the mental capacity of students enrolling in Animal Husbandry. About two-thirds of the Animal Husbandry students beginning their studies in 1956 were in the lowest half of the distribution of all freshman students entering the land-grant colleges and universities based on these data from 19 institutions. Approximately three-fourths of these students fell below the 60th percentile. This would seem to indicate that Animal Husbandry is not competing successfully with other curricula for the students of superior ability. It indicates further that we are probably being forced to pitch the level of our instruction to the average level of intelligence represented by students whose abilities place them approximately in the 40th percentile. It may also mean that we are doing a grave injustice to the 15 or 20 percent of our students who possess really outstanding mental capacity. The talent of our superior students in Animal Husbandry will not be given full opportunity for development if we permit them to work at less than their capacity. Insofar as we acquiesce in allowing our better students to take a curriculum pitched at the level of the majority of students coming into Animal Husbandry, we will be holding back the advancement of our profession. Here, it seems to me, we have some basis for reorientation or modification of our objectives in Animal Husbandry curricula.

In Figure 5 are data from 32 institutions, indicating the kinds of changes that have been made in Animal Husbandry curricula over the past 17 years. Numbers listed to the right of each bar give the range in semester hours required as reported by schools having the highest and lowest requirements.

There has been a slight reduction in Management and Production course requirements, a slight reduction in Market Types and Classes, and a reduction in Feeds and Feeding: considerable increase in Animal Nutrition, and moderate increases in Animal Breeding, Meats and other types of courses taught in Animal Husbandry Departments. We have increased the amount of Agronomy required, but have decreased the

amount of Agricultural Engineering, Dairy Industry and Farm Management. We have slightly increased the number of credit hours required in Veterinary Medicine subjects and decreased required courses in Agriculture offered in departments other than Animal Husbandry.

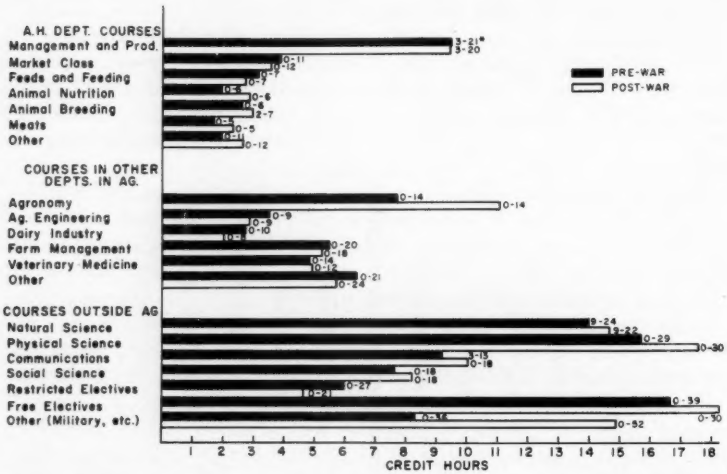

Figure 5. Curriculum Requirements in Animal Husbandry Pre-War and Post-War.

[a] States represented: Arizona, California, Colorado, Delaware, Florida, Iowa, Kansas, Louisiana, Maine, Michigan, Montana, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming.

[b] *Average* semester credit hours required:

Subject Matter Area	1940	1956
Management and Production	9.6	9.5
Market Types and Classes	3.97	3.63
Feeds and Feeding	3.22	2.81
Animal Nutrition	2.09	2.94
Animal Breeding	2.63	3.06
Meats	1.66	2.43
Other	2.16	2.69
Agronomy	7.75	11.13
Agricultural Engineering	3.56	2.97
Dairy Industry	2.78	2.16
Farm Management	5.59	5.28
Veterinary Medicine	4.88	4.97
Other	6.47	5.76
Natural Science	14.06	14.72
Physical Science	15.72	17.63
Communications	9.22	10.13
Social Science	7.69	8.25
Restricted Electives	6.06	4.63
Free Electives	16.66	18.42
Other	8.31	14.94

* Range in semester hours required as reported by schools having the highest and lowest requirements.

Considering course requirements outside of the School or College of Agriculture, we have increased the Natural Science requirements a little, and the Physical Science requirements by an average of 2 semester credits for the 32 institutions represented. We have increased Communications and Social Sciences somewhat, and have cut down on the numbers of Restricted Electives. Free Electives have been increased as well as certain of the required courses, such as Military Training.

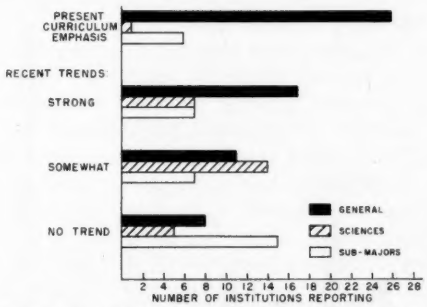

Figure 6. Current Animal Husbandry Curriculum and Trends.

[a] States reporting: Alabama, California, Colorado, Connecticut, Delaware, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Nebraska, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oregon, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin.

[b] Present emphasis and future trends by number of institutions:

	Present Emphasis	Trend		
		Strong	Somewhat	No Trend
Broad	26	17	11	8
Science Oriented	1	7	14	5
Sub-majors	6	7	7	15

By way of summary, Figure 5 tells us that we have increased Animal Nutrition, Animal Breeding and Meats instruction, whereas, we have reduced requirements in what might be considered the older classical types of Animal Husbandry instruction. There are few significant changes in the requirements in Departments of Agriculture other than Animal Husbandry, but some rather significant changes in courses required outside of the School or College of Agriculture, in that Natural and Physical Science requirements have been stepped up, as have also Communications and, to some extent, Social Science work.

We asked Heads of Animal Husbandry Departments all across the country about present curriculum emphasis—whether it was broad and general in nature so as to fit graduates reasonably well for a wide variety

of jobs; whether it was presently heavily weighted with chemistry, mathematics, physics and other natural and physical sciences; or whether it was split into several options or sub-majors such as sales, animal nutrition, animal breeding, farming, etc. We found that 26 of the 37 institutions reporting were presently emphasizing the broad general training which they believed would fit graduates reasonably well for a wide variety of jobs.

Only one institution reported that its present emphasis was heavily weighted with chemistry, mathematics, physics and other natural and physical sciences. Six institutions reported that they had split their curriculum into several majors, options or sub-majors such as sales, animal nutrition, animal breeding, farming, etc. Four institutions did not indicate the nature of their present curriculum emphasis.

We also asked Department Heads about recent curriculum trends, and we found that 17 institutions reported a strong trend for maintaining or moving toward the broad general type of education. Seven institutions indicated a strong tendency either to maintain or move in the direction of weighting their curriculum more heavily with chemistry, mathematics, physics and other natural or physical sciences. Seven institutions indicated a strong trend toward the practice of offering several options. Eleven institutions were moving somewhat in the direction of broad general training, and 14 reported there was some tendency toward moving in the direction of a curriculum heavily weighted with chemistry, mathematics, physics and other natural and physical sciences. Seven institutions indicated they were moving somewhat toward several options or sub-majors. A number of institutions reported no trends toward the three types of curricular programs described by this study.

As we consider these trends we note there are 21 out of 37 institutions moving strongly or somewhat toward weighting their curricula more heavily with chemistry, mathematics, physics and other natural and physical sciences. These schools appear to be very much in keeeping with the requirements of the future. Nonetheless, when we refer back to Figure 4, showing ACE scores, we wonder whether this trend provides a satisfactory answer, or whether we might serve our students better by offering several options or majors within the Animal Husbandry curriculum so that the stepped-up requirements for chemistry, mathematics, physics and other natural and physical sciences would be available for the more capable students, but would not necessarily be required of all students enrolled in Animal Husbandry. Perhaps it is significant that 14 of the institutions reporting indicated that they were trending in the direction of offering several options or sub-majors. Perhaps this is one of the alternatives that should receive serious consideration in the reorientation and modification of objectives in Animal Husbandry curricula. It seems clear that some such plan will be required if we are to face realistically the problem of working with a highly variable population, two-thirds of

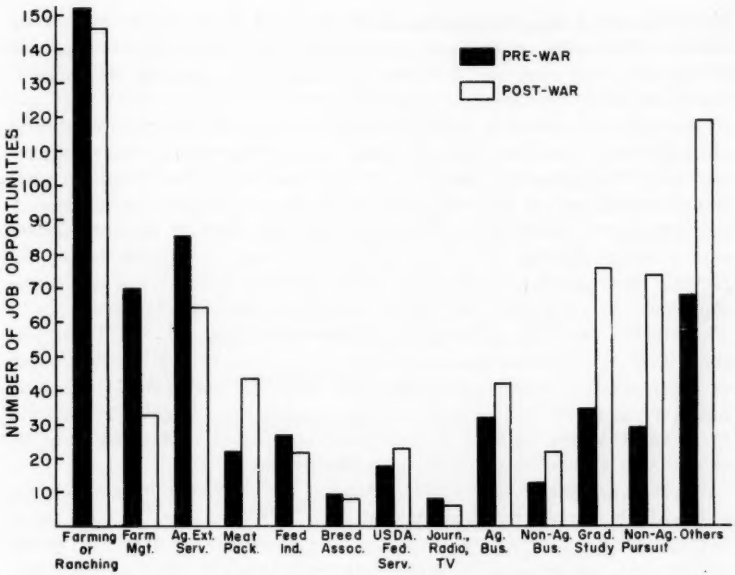

Figure 7. Job Opportunities, Pre-War and Post-War.

[a] States represented: California, Colorado, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, New York, North Carolina, Ohio, Oregon, South Carolina, Texas, Utah, Virginia, Washington, Wisconsin, Wyoming.

[b] Numbers entering each occupational area:

	1940	1956
Farming or Ranching	152	146
Farm Management	70	33
Agricultural Extension Service	85	64
Meat Packing	22	43
Feed Industry	27	22
Breed Association	8	7
USDA	17	23
Journalism	7	6
Agricultural Business	32	42
Non-Agricultural Business	13	22
Graduate Study	35	76
Non-Agricultural Pursuit	29	74
Others	67	119

which falls in the lowest 50% (based on ACE scores) of all-college student population. Perhaps we could best achieve our objectives if we were to utilize the talents of the majority of our students in Animal Husbandry for study in such options as sales, services, and farming, while at the same time offering the top 15 or 20% of our students professional training in animal nutrition, animal breeding, physiology and such other specialization as may be found in our Animal Husbandry programs.

Certainly it would be idle to discuss curriculum reorientation and modi-

fication without an examination of the fields of work which students in Animal Husbandry enter upon graduation. Data were secured from 22 institutions, indicating the first position of graduates in 1940–41 as compared to the first position of graduates in 1956–57.

These data revealed a slight decrease in numbers going into farming and ranching; a rather sizable decrease (only about half as many) going into farm management as was true 17 years earlier; fewer going into agricultural extension; nearly twice as many going into the meat packing industry; and a small decrease of those entering the feed industry. There was a slight decrease of those going into breed association work; an increase in those entering federal service with the USDA; and about the same number going into journalism, including radio and television work. The number entering agricultural business was increased, and there was some increase in those entering non-agricultural business. More than twice as many are now entering graduate study than was the case 17 years ago, and a great many more appear to be entering non-agricultural pursuits than was the case earlier. The category of "Others" including military service was also increased over the earlier years.

These data indicate that we are preparing our graduates for a somewhat different job market than was the case prior to World War II; large numbers are going into graduate study, and larger numbers are entering various agricultural businesses. There are increased numbers entering the meat packing industry. Perhaps these increases, when coupled with the somewhat decreased numbers entering the traditional fields of farming or ranching, farm management and the agricultural extension service, may dictate a reorientation and modification of objectives for at least some institutions.

Specifically, these data on changing job opportunities might prompt us to ask ourselves the question, "Could we do a better job if we were to offer perhaps 4 options or majors: (1) Livestock Production and Management, (2) Meats Technology, (3) Animal Industry Sales and Services, (4) Animal Science?"

No matter what changes we may make in our curricula, both the quality and numbers of students coming to us will likely be dependent to a degree upon how well our graduates compete with graduates of other disciplines in terms of salary. The questionnaire returns gave a picture of the salary situation as shown in Figure 8.

Comparing salaries for graduates during the year 1940–41 with those in 1956–57, we find that beginning Animal Husbandry graduates have been in a better competitive position than Liberal Arts graduates during both periods. Likewise, they have been at a disadvantage compared to Engineers during both periods. They have competed on equal terms with graduates from the other disciplines in Agriculture, but they have not drawn any premium over the other disciplines.

It is probably not surprising that Animal Husbandry graduates receive

smaller salaries than Engineers as they go onto their first jobs. This can be partially accounted for by the fact that many graduates in Animal Husbandry go back to the farm where their earnings are lower during the time they are becoming established in farming. In addition, Animal Husbandry graduates usually live in smaller communities where their actual take-home pay may be greater than that of Engineers living in large metropolitan centers and receiving considerably more gross income.

Perhaps if we were to market a better product in our graduates, we might be able to command salaries as high as those presently going to

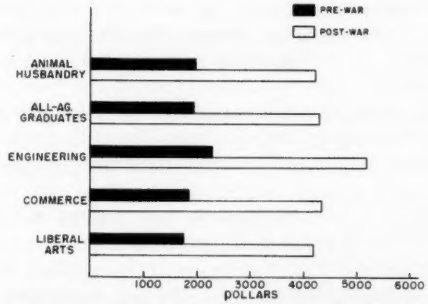

Figure 8. Average Yearly Beginning Salaries of Graduates, Pre-War and Post-War.

[a] States represented: Iowa, Kansas, Montana, New Jersey, Oregon, Vermont, Washington.

[b] Average salary by years:

	1940	1956
Animal Husbandry	$1980	$4260
All-Agriculture	1970	4310
Engineering	2230	5200
Commerce	1870	4380
Liberal Arts	1750	4210

Engineers. On the other hand, it may not be a matter of product, but a lack of aggressiveness on our part in placement activities. It is possible that we are not working closely enough with our friends in the packing industry, in the feed industry, breed associations, or the various other agricultural businesses and industries which utilize our Animal Husbandry graduates. By additional effort in placement activities we might be able to place our Animal Husbandry graduates in jobs which are now being filled by students graduating in commerce or in the liberal arts, or even in some of those positions presently being filled by Engineering graduates.

As professional Animal Husbandmen, we need to ask ourselves whether our undergraduate programs are sufficiently rigorous to turn out a product which warrants a higher price tag. At the same time, we should be equally concerned and equally willing to face up to a possible need for doing a better job of placement if this facet of our activities is in fact deterring

our graduates from receiving salaries comparable to those being paid to Engineering graduates.

If the data collected for this study from our land-grant institutions all across the country offer us some incentive for doing additional self-study and some critical self-evaluation, then it will have been well worth our time in discussing it at this annual meeting of the American Society of Animal Production. Certainly the last 20 years have been fruitful years for Animal Husbandry in terms of increasing size, in terms of professional development and in the achievement of a high order of academic respect. We have gained acclaim from our academic colleagues, and earned support from those who evaluate our work at both State and National levels. We have been accorded esteem from the public at large, and we can be very proud of the outstanding records of accomplishments made by our graduates.

Judged from almost any viewpoint, Animal Husbandry or Animal Science, if you choose to call it that, has made tremendous forward strides in the past two decades. Now, however, there are indications—only a few of which have been pointed up in this discussion—that certain reorientation and modification of objectives in our Animal Husbandry teaching programs might be desirable for the years ahead. How well we adjust to the demands of these changing times will very likely depend upon how vigorously we seek data for our guidance and upon how intelligently we interpret that data and upon our willingness to be guided by it in the modification and reorientation of our programs of instruction. The direction and rate of our progress during the next 20 years can be just as spectacular and as satisfying to those of us working in Animal Husbandry as have been the last 20 years, but to make it so will perhaps require more energy, more imagination and more willingness to adjust to changing conditions than have been required of us in the past.

ANIMAL HUSBANDRY GRADUATES IN INDUSTRY [1]

HERMAN W. SEINWERTH [2]

DR. HERRELL DE GRAFF of Cornell University in the September issue of County Agent made an interesting observation: "The industry-agriculture partnership that we hear so much about is nothing new. It's just been intensified to the point where we no longer can say just who is the farm worker!"

Dr. De Graff points out that despite sizable increases in population, the number of farm workers has been steadily decreasing. For example, in 1870 out of a total population of 40 million there were 8 million workers on the farms and ranches of the United States—a ratio of 1 to 5. Just 87 years later, in 1957, with an estimated total population of 170 million, there are still only 8 million workers on the farms and ranches—a ratio of about 1 to 22.

There is nothing startling or new about these figures. I quote them only to emphasize the dramatic change that has occurred in this specific area which the Land Grant Colleges were designed to serve. I have chosen to reiterate this emphasis because I believe that it has a tremendous impact upon the various phases of the problems this Society is considering at this 1957 annual meeting.

For, of all the implications this shift in population make-up has, probably the most significant from the standpoint of the institutions you folks represent is that fewer and fewer of the young men and women with whom you are working are returning to the environs from whence they came. In other words, more and more of them are seeking a livelihood away from the farm in industry, teaching and government situations.

This means a corresponding shift in the responsibilities of the Land Grant Colleges in preparing these young men and women for the opportunities which they will seek away from the farm. Such responsibilities fall particularly heavy upon men like yourselves. The training needed to fill these jobs in industry is materially different from that required to achieve the goals and objectives which have guided our colleges and universities in the past. In fact, I wonder if the vast changes taking place in the whole agricultural scene do not justify considerable modification in these goals and objectives even for those graduates who will still be returning to the farm.

I would like to explore the situation in the Meat Industry with reference to both opportunity and prerequisite training insofar as they concern

[1] Prepared for meeting of American Society of Animal Production, Chicago, Illinois, November 29, 1957.

[2] Industrial Relations Manager, Swift & Company, Chicago, Illinois.

men and women with an agricultural background trained in the broad field of animal husbandry.

By way of introduction, the Meat Industry is the third largest in the country in plant value of products. It is the connecting link between the producer of livestock and the consumer of meat. The very nature of the Meat Industry is such that it is quite stable and somewhat "depression proof!" It is characterized by large physical volume and small profit margins. There are more than 3,000 meat packing companies and 10,000 other smaller commercial slaughterers. These companies provide jobs for upwards of 300,000 people, about 100,000 of whom are salaried. A wide variety of job opportunities exists.

The American Meat Institute has accumulated for us information from other members of the industry in order to round out our own company experience in the fields of opportunity and prerequisite training for agricultural graduates. Information given here, therefore, will represent industry experience and views.

All of the companies surveyed by the American Meat Institute report an intensification of activity in contacting agricultural colleges for employment prospects. It is evident that this trend will continue in the years ahead. The critical need for better educated management prospects plus the potential benefits of agricultural training have undoubtedly caused this growing attention to your graduates. Industry needs exist in all ends of the business—marketing, sales, processing, research. In our own company, of the 50 colleges and universities whose graduates we have employed in large numbers, 28 are agricultural schools. Of all the college graduates in our employ currently, nearly 25% majored in some phase of agriculture. We, like most modern farmers and ranchers, have found that with the increasing application of scientific production and management methods comes the requirement of having access to more technical knowledge. This has meant seeking men trained in livestock production and management methods, in feeding and nutrition, in plant pathology and soils, in food technology and chemistry, and last, but by no means least, in that broad field of economics and marketing.

Let us consider some specific areas in which these adjustments touch on the field of employment opportunities for college graduates.

1. What starting jobs are available?

Agricultural graduates have started in many ends of our business. The first job is certainly not the last one. More often it serves as an entry point and a training position for the new employee. All too often we feel that the candidate for a new job places too much importance on the character of that starting job. Often he fails to fully appreciate that in a large organization particularly, we like to get the men started in some field where we have an opening so that we can then appraise and judge

his abilities and performance. Only in this way can we arrive at some judgment as to his future potentials in the fields of administration and management. There is plenty of opportunity for growth regardless of the entry point. At Swift, men who started in such diverse fields as livestock buying, accounting, by-products, sales, beef, provisions, poultry and processing are among our 50 meat processing plant managers.

Generally speaking, in the Meat Industry the new employee qualifies for more responsibility on an individual basis and only his initiative and his ability earn him the right to progress. The work and the hours may be hard—and they are—but the rewards will more than compensate in the long run.

Then, too, so many of the young men from the agricultural schools seem to have only one interest—livestock buying. Surely they have failed to appreciate all of the other opportunities that are open to them. By way of illustration let me name some of the specific areas toward which we move men trained in the field of animal husbandry; Beef Department, Lamb Department, Veal Department, Agricultural Research, Economic and Commodity Research, Dairy and Poultry Operations, Dairy and Poultry Sales, Feed Production and Sales, General Meat Sales, Hatchery Operations, Oil Mill Production and Sales, Plant Food Production and Sales, Livestock Buying, Provision Department, Time and Motion Study, and Research—just to name a few.

2. What starting salaries can the agricultural graduate expect to receive?

This, of course, is a difficult question to answer specifically because of the differences that exist in geographical location, job assignments, extent of specialization, and college degree. However, if you will permit me to generalize, I would place the starting salaries for agricultural graduates as ranging up to $100 per week with specialists and those with advanced degrees commanding even higher figures.

3. What are the rates of promotion for agricultural graduates?

This, too, is diffiult to answer specifically. So much depends on the individual. Given the ability, initiative and the desire, progress may be very rapid. With the very evident need in our industry as well as in industry generally for better qualified management people, the only limiting factor to the rapidity of promotion is the individual himself.

Approaching this question from another standpoint, one of our younger vice presidents became an officer in 17 years from the time he went to work. Some of our unit managers have attained appointment in as little as five years. Some of our general sales and commercial people have taken only a couple of years to reach an excellent level of earnings and performance. Again all of this is achieved on the basis of individual performance rather than through formalized group management development programs.

Whenever one considers rate of promotion, he must of necessity bear in mind that all people do not progress to the same heights. In most organizations there are many people who are performing quite satisfactorily and who are quite happy and well adjusted but who have not reached the top rung of the ladder. A rather trite way of saying this is that not all of us can be chiefs—we must have some Indians.

4. What are the ultimate levels to be reached by agricultural graduates?

One of the companies surveyed reported that currently they have two vice presidents, six department heads, and five branch house managers all of whom have an agricultural background. In Swift & Company animal husbandry graduates occupy such positions as president, vice president, plant manager, plant superintendent, department head and supervisor.

We have long held the belief that we can raise better men than we can hire. This policy has had a very definite bearing upon the ultimate levels reached by our people as well as the average length of service. A recent survey of over 2,000 Swift & Company executives showed that 95% of these men started at the bottom of the ladder. Seventy-three percent were employed under 25 years of age.

5. How many agricultural graduates are employed in the Meat Industry?

I have been unable to secure any meaningful figures in this connection, other than the general trends in the observations made earlier. However, to give you a quantitative indication in our own company we may hire as many as 300 or more college graduates annually, and a good proportion of these are from agricultural schools. Further, we know from pure observation that a high percentage of the agricultural graduates are men who have been in animal husbandry schools.

Now, what are the requirements and training necessary to fill a number of the jobs which have been mentioned in the foregoing?

I think it is only fair to observe that the Land Grant Colleges have by law, in past years, emphasized the production and marketing of agricultural commodities from the viewpoint of the agricultural producer. This has meant training men to produce and market the products of America's farms and ranches. As a consequence much of the teaching and curricula in the agricultural departments is still directed toward such questions as increasing productivity per acre, per animal, per man hour, etc. Likewise considerable emphasis has been given to discovering and teaching farmers the best and most efficient means of marketing their livestock and grain and feed crops.

In recent years there has been a sharp acceleration in the number of your graduates who have had to seek employment in industry rather than returning to the farms and ranches.

From those institutions that have been unable or have not seen fit to

change their curricula to more fully prepare their agricultural graduates to enter some phase of industry, we receive graduates trained in production and marketing from farm to market, but lacking a sufficient background in industrial processing and an understanding of the workings of the market place.

I do not, however, want to seem entirely negative on this question. A number of institutions have already made some appropriate adjustments. I refer for example to the schools that have added meats courses to the animal husbandry curriculum. This has, in most cases, been done over the past 10 to 20 years. In addition, we know of a number of schools who have added new course work to their agricultural curriculum, which courses add materially to the students' background for an entree to an agricultural-related industry. In this connection, emphasis has been given in recent years to such fields as packaging, food technology, marketing consumer goods, and research methods. Further than this, the addition of placement bureaus and counseling service for students has played an important part in directing the undergraduate curriculum toward the desired end.

It seems apparent that students will need to be guided in the appropriate areas of interests and adjustments made in their curriculum accordingly. It is quite probable that such subjects as food technology or engineering may be increasingly important to those who wish to prepare themselves for work in a number of fields related to agriculture. As processing operations become more technical and scientific in character, there will be a greater use of college trained men in processing assignments, and it is in this connection that some engineering and science courses may pay real dividends to the individuals involved.

We cannot overemphasize the desirability of training to improve the ability of the individual to express himself. All too often we observe an individual who has been well trained in specific functional areas but who is grossly inadequate in expressing himself and in communicating with others. Communications is management in action. Courses such as public speaking, journalism, business writing and even grammar and spelling should not be overlooked.

Extra curricular activities are also helpful in developing communications effectiveness and, in addition, usually make a real contribution in broadening the personality.

The field of sales in industry is so large in itself that it would be difficult to visualize our Land Grant Colleges overlooking this basic subject. Yet we are almost continually amazed at the lack of interest in and preparation for sales work in a great many agricultural colleges and universities.

Then there is the matter of specialization. Too much can be a handicap. Industry wants men who are well versed in the fundamentals of several sciences and who are imaginative and adaptable enough to apply these

fundamentals in such fields as processing, marketing, research activities, and possibly later to management. Versatility is always of value.

Another subject mentioned frequently by various companies in the industry as being highly desirable is agricultural economics. Several companies reported that those agricultural graduates who had majored in agricultural economics seem to have made very rapid progress. This may have been because of specific opportunities present at the time in these companies, but the observation is nonetheless an interesting one.

These are a few—but major—points which seem to suggest themselves immediately in the subject under discussion. They may well deserve your amplification and consideration in the problem of preparing the animal husbandry graduate for a career in industry. Just how your curriculum can best be adjusted to provide for the needs suggested here is certainly something that is in your province to determine. I am not an expert in the field of education and would not presume to suggest how the objectives suggested here could best be attained.

Summarizing, it should be considered first of all that there are wide opportunities in the Meat Industry for agricultural graduates, trained in the broad field of animal husbandry and/or meats. Certainly there will be no lack of attractive job opportunities available for those who have the necessary training and attributes.

Secondly, the educational requirements to best fit the agricultural graduate for a job in industry vary significantly from those needed to best prepare him for a job on the farm. As these needs are taken into account in adjusting the courses of study, and in counseling with the young men entering such college programs, we should see the emergence of a group of men who would be admirably prepared to make a real contribution, not only to industry but to agriculture generally. I say this because the better trained and qualified the men who are marketing America's agricultural products, the better are likely to be the results, both in industrial performance and on America's farms and ranches.

Considering the very fine success we have had in utilizing agricultural graduates, we look forward to continuing our close relationship with you and the many others who are associated with you in this basic educational area of animal husbandry.

DETERMINATION OF SPECIFIC GRAVITY BY AIR DISPLACEMENT [1]

J. A. Liuzzo,[2] E. P. Reineke and A. M. Pearson
Michigan Agricultural Experiment Station,[3] East Lansing

ONE of the major problems in studying growth of domestic animals is to obtain an accurate measure of the composition of body gains. A method of determining body composition would be useful in selecting breeding stock as well as in measuring the composition of gains of animals on nutritional or physiological experiments. The water displacement method of determining body volume based on the Archimedean principle dates back to the 18th century. Modifications have made this technique applicable in determining specific gravity which can be used to evaluate the status of body components in human beings and animal carcasses (Rathbun and Pace, 1945; Brozek and Keys, 1950–1951; Brown *et al.*, 1951; Kraybill *et al.*, 1953; Whiteman *et al.*, 1953; Pearson *et al.*, 1956a, 1956b; Price, *et al.*, 1957). However, this method is not applicable to living animals.

A helium dilution technique has been employed in measuring the body volume of human beings (Siri, 1953, 1955, 1956) and live animals (Walser and Stein, 1953). Determination of body volume by measurement of the air displaced has been investigated by several workers in Germany (Pfaundler, 1916; Kohlrausch, 1929a, 1929b; Pfleiderer, 1929; Bohnenkamp and Schmäh, 1931; Noyons and Jongbloed, 1935; Jongbloed and Noyons, 1938). The major criticisms of these techniques are that they are time consuming and tedious. These investigators did not relate their calculated values with actual chemical composition of the body. Wedgwood and coworkers (1953a, 1953b) have also reported the construction of an apparatus designed to measure body volume by air displacement but the method has not been described in detail, nor are results reported in the literature.

The object of this project was to develop a more practical technique for determination of specific gravity by air displacement in living animals and to correlate the values obtained with the major body constituents.

Experimental Procedure

Experiment I. In this investigation, the method employed was based on the theory that if an animal is placed in a closed chamber, the gas space within the chamber will be reduced in proportion to the body volume of

[1] Journal Article No. 2010, Michigan Agricultural Experiment Station.
[2] This is a portion of research completed for the Ph.D. thesis by the senior author.
[3] Departments of Animal Husbandry, and Physiology and Pharmacology.

the animal. Measurement of the change in volume is based on the application of Boyles' law which states that the volume of a given mass of gas at a constant temperature is inversely proportional to the pressure to which it is subjected, or $P_1V_1=P_2V_2$ where P=pressure and V=volume. From the pressure-volume relationships between the chamber in which the animal is placed and a connected chamber of known size, where the two are subjected to varying pressures, the air displaced, and consequently the body volume of the animal can be computed.

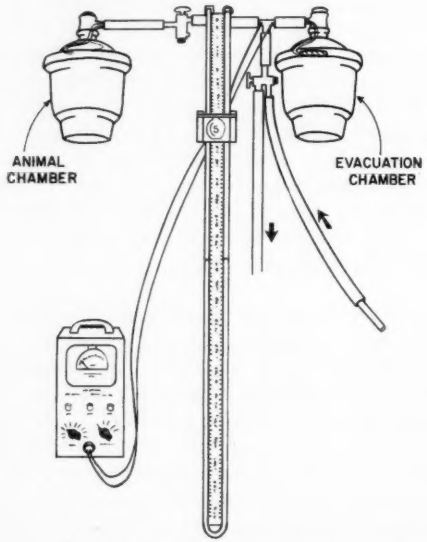

Figure 1. The apparatus designed to measure body volume by air displacement.

The purposes of this experiment were twofold, (1) the development of a practical apparatus for measuring body volume, and (2) determination of the relationship of specific gravity values thus obtained with body composition. The original apparatus (figure 1) was constructed from 2 desiccator jars of approximately 2600 ml. capacity, which were connected to each other, to a mercury manometer, to a vacuum pump and to a spirometer by a suitable arrangement of stopcocks. The spirometer was used to saturate incoming air with water vapor to avoid changes in vapor pressure. Temperature changes in the evacuation chamber were measured with a thermistor.

The steps involved in using the apparatus for determining body volumes were as follows: (1) The mercury levels of the manometer were read while the instrument was "at rest" (levels read on both sides to minimize errors),

(2) a vacuum of 320±10 mm. of Hg. was drawn on the evacuation chamber (this level was selected to permit sufficiently accurate differential readings and yet not subject the animal to excessive vacuum), (3) after recording the temperature in this chamber and reading the mercury level, equalization between the two chambers was allowed to occur by turning the stopcock closest to the animal chamber, (4) thirty seconds were allowed for equalization after which time the temperature was recorded and the mercury level re-read. The initial manometer reading was designated as P_0, the reading before equalization as P_1, and the reading after equalization as P_2. The values obtained were incorporated into formula (1) which is a rearrangement of the basic gas law equations.

$$V_2=\frac{P_1-P_2}{P_2}\left(V_1\frac{273}{273+\triangle T}\right) \quad (1)$$

where V_2=volume of the empty animal chamber.
V_1=known volume of the evacuation chamber.
$\triangle T$=temperature change in the evacuation chamber.

The procedure was repeated with the animal in the chamber to determine the volume of the animal chamber while occupied (V_3). Replicate determinations were made for V_2 and V_3 with each animal. The average of these values was incorporated into formula (2) for determining the volume occupied by the animal.

$$\text{Volume occupied by the animal}=V_2-V_3. \quad (2)$$

The specific gravity of the animal was determined by formula (3).

$$\text{Specific gravity}=\frac{\text{weight of animal in air}}{\text{volume of animal}} \quad (3)$$

Following preliminary experiments, which indicated the value of the method in measuring the volume of innate objects, 23 live female guinea pigs were used to test its applicability. The guinea pigs, weighing from 507 to 1,064 gm., were deprived of food and water for 24 and 12 hours, respectively, prior to the determinations. The hair was clipped from the animals to reduce weighing errors in the subsequent water specific gravity determination. Specific gravity was determined by the air displacement procedure using a P_1 value of approximately 320 mm. of Hg. The animals were then sacrificed and eviscerated of all organs except the kidneys and surrounding fatty tissues. In order to check the validity of the air displacement method, specific gravity of the eviscerated carcasses was determined by the water displacement method of Rathbun and Pace (1945). Carcasses were ground and mixed, then frozen and stored at −20° F. in glass jars. Body fat, water, protein and ash of the carcasses were determined by A.O.A.C. methods (1955). These values were correlated with air and water specific gravities and regression lines were calculated according to methods described by Snedecor (1946).

Experiment II. The purposes of this experiment were to ascertain the feasibility of using a smaller negative pressure for the body volume deter-

minations than has been used in Experiment 1 and to investigate the influence of visceral composition upon specific gravity of the intact animal. At the small negative pressures employed in this experiment (120 and 220 mm. of Hg.), changes in dead-space of the differential manometer used in Experiment 1 would become too large to be disregarded. Therefore, the manometer tube was reconstructed to record readings at constant volume. A side-arm was fused to the base of the manometer and connected to a leveling bulb with rubber tubing. This mercury reservoir was raised or lowered to obtain all readings at the same point on the inner arm of the manometer. The constant volume technique at 120±10 mm. of Hg. in the evacuation chamber was adopted for this experiment, after preliminary

TABLE 1. SUMMARY OF CORRELATION COEFFICIENTS [a]

	Water specific gravity		Air specific gravity	
Body component	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Fat, %	—0.99	—0.97	—0.70	—0.82
Water, %	0.97	0.95	0.67	0.81
Protein, %	0.96	0.87	0.68	0.72
Ash, %	0.65	0.69	0.56	0.72

[a] All values signficant at the 1% level.

determinations of V_2 at negative pressures of 120 and 220 mm. of Hg. showed the constant volume technique to be more reliable.

Twenty-five female guinea pigs ranging in weight from 512 to 1284 gm. were subjected to body volume determinations by air displacement at a P_1 value of 120 mm. of Hg. The water displacement measurements and carcass composition studies were conducted as in the previous experiment. The viscera, including the contents of the digestive tract of 23 guinea pigs were ground and analyzed in order to determine the influence of visceral composition upon live animal specific gravity.

Results and Discussion

In Experiment 1, the correlation coefficients (table 1) for the air specific gravity method were —.70, 0.67, 0.68 and 0.56 for percent fat, water, protein and ash, respectively, in the eviscerated carcass. Thus, variations in air specific gravity accounted for approximately 49, 45, 46 and 31% of the variation in fat, water, protein and ash. In Experiment 2, the correlation coefficients (table 1) for the air specific gravity method were —.82, 0.81, 0.72 and 0.72 for percent fat, water, protein and ash in the eviscerated carcass, respectively. Thus, variations in air specific gravity accounted for approximately 67% of the variation in fat, 66% for water, and 52% for protein and ash. These results indicate that it was advantageous to employ a small negative pressure and the constant volume technique of mano-

meteric measurement. A comparison of the relationships of specific gravity obtained by the water displacement method with those obtained by air specific gravity show the former method was more closely correlated with each chemical component. It is apparent that the air displacement method was less accurate.

In the interpretation of these results, exact agreement should not be expected between the air and water methods since the air method is based on the intact animal, which includes all viscera and the contents of the digestive tract, whereas the water displacement method must necessarily be made on the eviscerated carcass. In the air displacement method, pressure changes would be distributed uniformly throughout the chamber and respiratory tract of the animal, thereby correcting for the air spaces of the lungs, trachea and nasal passages automatically. Since it is impossible to make such corrections with the water displacement method, the eviscerated carcass was used. Although the work of Behnke *et al.* (1942) has verified the relationship between water specific gravity and chemical components, only a limited amount of work using a small number of animals has been reported on the air displacement method and body components (Kohlrausch, 1929a, 1929b).

Chemical analysis of the viscera in Experiment 2 made it possible to assess the variation due to the contents of the digestive tract and composition of the viscera. The correlation coefficient of air displacement specific gravity and percent fat on the whole animal basis (eviscerated carcass plus viscera) was —.73 as compared to —.79 for air specific gravity and the eviscerated carcass. Thus, the composition of the viscera did not appear to be an additional source of error in the air displacement method, otherwise the relationship of specific gravity and percent fat on the whole body basis would have been higher than that for specific gravity and percent fat of the eviscerated carcass. This is in accordance with the results of Rathbun and Pace (1945) using the water specific gravity method.

The influx of warm air from the animal chamber into the evacuation chamber before recording P_2, caused a measurable temperature change in the latter chamber. However, temperature variations in the animal chamber are of little importance since this chamber is left open to the atmosphere except for 30 seconds required for equalization. In order to estimate improvement in accuracy obtained by correcting V_2 and V_3 for temperature, correlation coefficients were calculated for water and air specific gravities with volumes corrected and uncorrected for temperature. The correlation coefficient for the specific gravity calculated from volumes corrected for temperature was significant at the 1% level (0.74); however, when not corrected the correlation (0.43) was significant at only the 5% level. Therefore, it is apparent that correcting volumes for temperature resulted in greater accuracy.

A further source of variation would enter into the measurement, if the degree of saturation of air in the chamber were not controlled. This variable

was eliminated by keeping the air saturated in both chambers. It might be questioned whether changes in gas volume due to respiratory gas exchange would introduce significant errors. Inasmuch as the animal chamber was connected into the closed system for only about 30 seconds to obtain P_2, the calculated error due to this source would not exceed 0.8 ml. in animals with normal metabolic rate and RQ in the usual range.

In Experiment 1, the first of four replicates of V_3 for each animal was consistently higher than the following three volumes. The average of the 23 first V_3 values was 1948.3 ml. as compared to 1921.4, 1921.5 and 1921.3 ml., respectively, for the succeeding three. A "t value" of 4.87 indicates the difference between the first and subsequent values to be significant at the 1% level. This may be due to the partial removal of gases from the animal's blood, body fluids or digestive tract as a result of exposure to negative pressure. Once this increment of gas was removed, it apparently was not restored appreciably during the time between measurements, as evidenced by the close agreement between the 3 succeeding replicates. Therefore, the first value was discarded in determining the average volume of the chamber. In 37 average volumes obtained for the unoccupied animal chamber over a period of 4½ months, the standard error was ± 3.14 ml.

In Experiment 2, it was observed that the accuracy of specific gravity results could be improved if body volumes were all determined by using one standard V_2 value. Formerly, the value employed was the average of four V_2 determinations for each guinea pig. The correlation coefficient for water and air specific gravities was 0.74 when the values were calculated by the earlier method. However, the coefficient rose to 0.83 when the standard V_2 value was employed. This standard value (2642.7 ± 2.01 ml.) was the mean of thirty-two V_2 determinations recorded over a period of one week. The air specific gravity values which were correlated with body components (table 1) in Experiment 2 were derived by using the standard V_2 value.

This method of predicting body composition approaches the accuracy of the various methods now employed in determining fatness of live animals. The method is much faster than the technique reported by Pfaundler (1916) who used infant cadavers for his determinations. His readings for body volume determinations required 1½ hrs., which is too time consuming for living subjects. Other workers have investigated air displacement techniques but the methods used have also been time consuming and tedious (Bohnenkamp and Schmäh, 1931; Jongbloed and Nyons, 1938; Pfleiderer, 1929). Furthermore, there has been little attempt to relate body volumes thus obtained with body composition. The present technique requires less time than earlier methods. The calculations and actual determinations are less tedious, and the specific gravity values obtained have been found to be closely correlated with body composition.

Currently, a large animal chamber is being constructed at this Station in order to conduct similar studies with market weight hogs, for it is be-

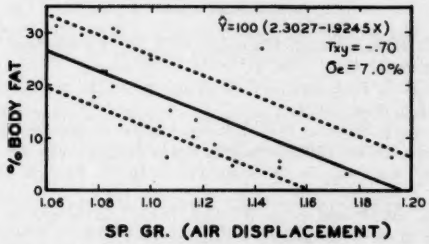

Figure 2. Regression line of air specific gravity and % body fat in Experiment 1.

lieved that the technique reported shows sufficient promise to merit further investigation in its application to the measurement of specific gravity of live farm animals.

Summary

A technique was developed for the determination of body volumes of living animals by air displacement. Two experiments were reported in which body volumes were determined at two levels of negative pressure. In the first experiment, specific gravity of 23 adult female guinea pigs, calculated from body volumes determined at a total vacuum of 320 mm. of Hg., were correlated with carcass fat, water, protein and ash. The correlation coefficients were —.70, 0.67, 0.68 and 0.58, respectively. In the second experiment, body volumes of 25 female guinea pigs were determined at a total vacuum of 120 mm. of Hg., employing a constant volume method of manometeric measurement. Correlation coefficients for air specific gravities thus obtained and carcass fat, water, protein and ash were —.82, 0.81, 0.72 and 0.72, respectively. Other factors which influenced the accuracy of the body volume determinations were changes in chamber temperature and humidity. Variations due to the contents of the digestive tract and composition of the viscera did not significantly alter the relationship between air specific gravity and carcass composition.

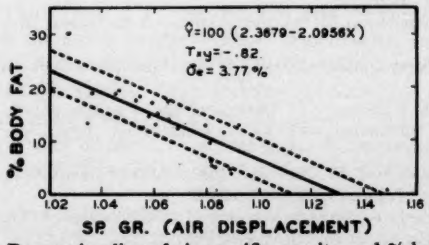

Figure 3. Regression line of air specific gravity and % body fat in Experiment 2.

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ADJUSTMENT OF MILK LAMB WEANING WEIGHTS TO A STANDARD AGE [1]

BRUCE L. WARWICK [2] AND T. C. CARTWRIGHT [3]

Texas Agricultural Experiment Station

IN the normal management of lambs used in a research flock, weaning is scheduled for one or more fixed dates rather than at a fixed age. This results in considerable variation in age, necessitating the use of some method of estimating what the weight would have been at the standard age selected. The same problem presents itself to the breeder if he attempts to use weaning weights for selection. Where only one weight at or near weaning is taken, there are several methods which may be used:

(1) Weight per day of age $\times$ the standard age in days.
(2) Gain per day from birth $\times$ the standard age in days, plus birth weight.
(3) Adjustment to standard age by use of regression of weight on age (data from group weighed).
(4) Correction factors based on previous data for each sex, type of raising, and age in days used as constants $\times$ weight or as added increments. When the animals may be weighed 2 or more times, weights may be adjusted by use of the gain during the period.
(5) By interpolation if the weigh dates bracket the standard age date for the particular animal, or by extrapolation if the standard age is not bracketed.
(6) By use of the actual weights taken at the nearest weigh date to the standard age date for the particular animal.

Of these methods, adjustment by regression has been used regularly at the Western Sheep Breeding Laboratory (Hazel and Terrill, 1945) where the lambs were dropped in about a 45-day period. They were run in three age group bands and weaned on three successive dates so that each band was weighed when the average age approached the standard age. Phillips and Brier (1940) described a method for use with certain mutton breeds which they called the age intercept method, which was a combination of the lamb's weight and an adjustment factor based on average growth for the period between the standard age and the age intercept for the breed, sex, litter size and month of birth based on previous data. Frequent use is made of gain per day of age to weaning without computing an adjusted weaning weight. Weight per day of age was used at this station in earlier

[1] This work is part of S-29 Regional Milk-fat lamb project, which is cooperative with the Southern State Experiment Stations, and the U. S. Dept. of Agriculture.
[2] Animal Husbandman and Geneticist, McGregor, Texas.
[3] Associate Animal Husbandman and Geneticist, McGregor, Texas.

years but the method was replaced by the use of adjustment based on weights bracketing the date the lamb became 120 days of age. The use of corrections based on previous data (Phillips and Brier, 1940) is subject to discrepancies because the seasonal conditions vary widely (Warwick and Cartwright, 1957) and the corrections represent average effects, rather than applying specifically to the particular season and animal.

Experimental Procedure

Lambs born in the Experiment Station flock at McGregor, Texas from Oct. 8 to Dec. 9, 1956 were used to compare methods. The lambs and their mothers were self-fed a ground high-roughage ration until winter oats pas-

TABLE 1. LAMBS AVERAGED BY 120-DAY ACTUAL WEIGHTS AND WEIGHTS ON APRIL 10, 1957

Breed, strain or cross	No. of lambs	Actual wt. 120 days age	4–10–57 weight
		lb.	lb.
119	10	72	104
443D	4	67	96
Ramb.	49	66	90
443R	11	63	91
404	25	63	85
449	16	61	79
All	115	65	90

ture was available the last part of November. No creep was used, and after oats pasture became available no other feed was fed except temporarily during inclement weather. The lambs used were as indicated in table 1.

Cross 119 is F_1 Rambouillet ewes x a Suffolk ram. Strain 443D is from crossing Rambouillet ewes x Dorset Horn rams and back-crossing to the Dorset. They represent over 50% Dorset Horn breeding. Strain 443R was started together with strain 443D but has been bred back to Rambouillets and is less than 50% Dorset. Strain 404 originated from crossing Rambouillet ewes x Romney Marsh rams followed by back-crossing to the Rambouillet, and breeding *inter se*. They are less than 5% Romney. Strain 449 originated from crossing Rambouillet ewes x Wild Mouflon (*Ovis musimon*) rams which was followed by backcrossing and *inter se* breeding.

All of the lambs were weighed (1) on the exact day that they reached 120 days of age, (2) when the oldest lamb was approximately 90 days of age and (3) approximately each week thereafter until the youngest reached 120 days of age. These weights, taken to the nearest pound, were used to compute adjusted weights by all methods mentioned except those involving predetermined correction factors. Intervals of 1, 2, 3 and 4 weeks were

designated to use with the different methods. Simple correlations between the actual 120-day weights and adjusted 120-day weights by each method and interval were computed as "over-all" correlations, respectively. Because analysis of variance of actual 120-day weight showed significant differences attributable to sex and type of raising (Warwick and Cartwright, 1957) each animal was coded by the schedule shown in table 2, and correlations were also computed on the "within sex and type of raising" basis. Examination of the data indicated that each breed, cross or strain started lambing within about one week and that there was no apparent difference in lambing pattern with respect to dates.

TABLE 2. CODES USED TO GROUP LAMBS

Code	Sex	Type of raising
1	Male	Single raised as single.
2	Male	Twin but raised as single.
3	Male	Twin raised as twin.
4	Castrated male	Single raised as single.
5	Castrated male	Twin but raised as single.
6	Castrated male	Twin raised as twin.
7	Female	Single raised as single.
8	Female	Twin but raised as single.
9	Female	Twin raised as twin.

Table 3 shows the average differences between actual 120-day weight and weight adjusted by the respective method. In five instances the number of animals is 105 or less because some of the ram lambs had been weaned earlier than one of the dates involved, making their weights unusable. For the method involving 113 animals, two were unsuitable for the particular method because of age.

Results and Discussion

The "within group" correlation coefficients between the actual 120-day and the respective adjusted weights by each method and time interval are shown in table 3. The "overall" coefficients are not shown since almost all were identical to the "within" values and none varied more than 0.01. These coefficients are all statistically highly significant and indicate that most of the adjusted weights are good estimates of the actual 120-day weight.

It will be seen from these data that the best estimates are apparently from bracketed weights a week apart. However, there are fifteen other methods which closely approached (correlation coefficients of 0.98 and 0.97) the reliability of the week apart bracket method. These indicate a choice between actual weights, weight per day of age, gain per day from birth, extrapolation of gain and bracketed weights at different intervals

TABLE 3. AVERAGES OF, DIFFERENCES BETWEEN AND THE CORRELATION COEFFICIENTS BETWEEN ADJUSTED AND ACTUAL 120-DAY WEIGHTS (LB.)

Method of calculating adjusted weight	N	Av. Actual Wt.	Av. Adj. Wt.	Av. Diff.	Largest diff. above actual	Largest diff. below actual	Correlation between actual and adjusted wt.[a]
Weight per day age:							
1. Weight per day of age (times 120) based on the first weekly weight taken after the individual lamb reached 120 days of age.	115	64.7	62.5	2.2	4.1	9.5	0.97
2. Weight per day of age (times 120) based on the first weekly weight taken before the individual lamb reached 120 days of age.	115	64.7	63.9	.8	5.4	6.1	.97
3. Weight per day of age (times 120) from weight taken on the date nearest 120 days of age, based on weights taken every two weeks.	115	64.7	62.7	2.0	5.4	7.7	.96
4. Same as (3) except weights taken every three weeks were used.	115	64.7	63.1	1.6	5.2	9.5	.97
5. Same as (3) except weights taken every four weeks were used.	115	64.7	63.2	1.5	6.9	6.5	.98
6. Same as (3) except weights taken on date flock averaged 87 days of age were used.	115	64.7	71.9	—7.2	3.4	20.3	.96
7. Same as (3) except weights taken on date flock averaged 102 days of age were used.	115	64.7	65.1	.4	7.3	8.3	.96
8. Same as (3) except weights taken on date flock averaged 121 days of age.	105	64.7	63.0	1.7	4.7	9.5	.97
Gain from day of birth:							
9. Same as (1) except gain from birth was used.	115	64.7	62.5	2.2	6.0	9.0	.97
10. Same as (2) except gain from birth was used.	115	64.7	63.7	1.0	5.0	6.3	.97
Interpolation or extrapolation:							
11. Weight taken at first weekly weighing before the lamb reached 120 days of age plus an amount prorated from the gain to the next weekly weighing to correspond to 120 days of age.	115	64.7	63.2	1.5	3.0	6.0	.98
12. Same as (11) except weights taken every two weeks were used.	88	64.1	62.4	1.7	5.3	5.5	.98
13. Same as (11) except weights taken every three weeks were used.	86	65.0	63.6	1.4	3.4	5.9	.98
14. Same as (11) except weights taken every four weeks were used.	75	62.8	61.2	1.5	3.6	5.7	.98

TABLE 3—(*Continued*)

15. Weight taken at first weekly weighing before lamb reached 120 days of age plus an amount prorated from the gain of the lamb during the week preceding the week during which the lamb reached 120 days of age.	115	64.7	64.1	.7	10.4	10.0	.95
16. Same as (15) except weights taken every two weeks were used.	115	64.7	63.7	1.0	8.4	9.0	.95
17. Same as (15) except weights taken every three weeks were used.	115	64.7	63.5	1.2	7.0	7.0	.96
18. Same as (15) except weights taken every four weeks were used.	115	64.7	62.7	2.0	4.3	7.1	.97
Regression:							
19. Weight taken when flock averaged 87 days of age.	115	64.7	51.9	12.8	—	3.2	.93
20. Wt. taken when flock av. 102 days of age.	115	64.7	55.2	9.5	—	3.8	.94
21. Wt. taken when flock av. 121 days of age.	105	62.8	61.9	.9	5.1	15.9	.95
22. Two weights taken: (1) when older ½ of flock averaged 120 days and (2) when younger ½ averaged 120 days of age so that maximum age difference in either group was 15 days (two b values used).	113	64.7	63.0	2.1	4.0	9.0	.98
Actual weights:							
23. Weight (actual) taken at weekly weighings that most nearly coincided with individual lambs 120 days of age.	115	64.7	63.1	1.6	3.0	6.0	.98
24. Same as (23) except based on weights taken every two weeks.	115	64.7	62.9	1.8	5.0	9.0	.97
25. Same as (23) exept based on weights taken every three weeks.	115	64.7	63.3	1.4	10.0	9.0	.93
26. Same as (23) except based on weights taken every four weeks.	115	64.7	62.0	2.7	7.0	8.0	.95

[a] Coefficient computed within groups. Confidence limits, P=0.99, for each value are: r=0.99, 0.984 and 0.994 r=.98, .968 to .988; r=.97, .952 and .981; r=.96, .936 and .975; r=.95, .920 and .969; r=.94, .904 and .963; r=.93, .888 to .956.

and the regression method. Weights adjusted by use of regression coefficients where only one weight is taken for the entire lamb crop are much less closely correlated with the actual 120-day weights than are most of the other adjusted weights. This emphasizes the caution necessary in limiting projections of regression estimates. Any one of the methods checked indicates a close enough estimate to be useful, and those with correlations of 0.97 to 0.99 offer a wide choice with a high degree of reliability.

With milk lamb production on winter oats pasture, some precocious ram lambs have become sexually mature at 120 to 130 days of age. For this reason, it is desirable to wean all ram lambs at weigh days before they reach 120 days of age. This may be done and valid, adjusted 120-day weights computed by extrapolation if the interval between weigh days is not more than 3 weeks or by the use of weight per day of age if within 3 weeks of the date the lamb will be 120 days of age.

Summary

Weaning weights of lambs adjusted by six types of procedures have been compared with the actual weights of 115 lambs at 120 days of age; varying the procedure in each type with respect to time or interval, brought the number of methods compared to 26. The correlation coefficients varied from 0.99 by the use of bracketing weights of one week interval to the lowest of 0.94 by use of regression using one flock weight taken at earlier ages, and by actual weight at nearest weigh day with intervals of three weeks. The results were very similar when the correlations were computed "over-all" to those computed "within sex and type of raising." All fifty-two correlations were statistically significant, and offer a wide choice of valid methods.

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EFFECT OF CALVING DATE ON SUBSEQUENT CALVING PERFORMANCE

MARTIN J. BURRIS [1] AND B. M. PRIODE

Virginia Agricultural Experiment Station and U. S. Department of Agriculture [2]

A HIGH calf crop percentage is an essential part of the profitable beef cattle enterprise. Many factors influence the ability of a cow to calf in a given year and successively over a number of years. This paper was prepared for the purpose of showing the association between the performance of individual cows in consecutive breeding seasons. An attempt was made to determine the amount of variation in calving performance which can be accounted for by previous calving performance.

Materials and Methods

Calving records of the research herds of Angus, Hereford and Shorthorn cattle at the Beef Cattle Research Station at Front Royal, Virginia, from 1950 to 1957 were used. Calving dates were coded as day of the year (January 1=1, April 10=100, etc.) The calving season was usually restricted by limiting the breeding season to 90 days. However, there were exceptions to this practice. At the end of the breeding season in 1951 a Hereford bull was found to be sterile and was replaced at this time by another Hereford bull which bred the cows in this herd after the regular breeding season. Bred cows brought into the herd were allowed to calve and placed in pastures with the bull during the next regular breeding season. Previous to 1953 breeding season, bulls were turned out with cows on May 1. In 1953 and subsequent years the bulls were turned out with cows on April 1 or shortly thereafter. Cows were assigned at random within breed to the breeding herds in nearly all cases and in no instance was assignment to breeding herds based on calving date.

Calving dates were secured by patrolling pastures every 24 hours and ear tagging and identifying all new calves. Very little selection among cows was practiced in this herd since a primary objective during these years was to increase the total number of females in the breeding herd. Average age of Shorthorn and Hereford cows was greater than for Angus cows, and cow mortality was also higher in the Shorthorn and Hereford herds.

Three determinations were made from these calving records to measure the effect of date of calving on subsequent calving performances. The determinations include: (1) Percent of cows calving each 20-day period during the calving season which failed to calve the following year, (2)

[1] Present address, State Experiment Stations Division, A.R.S., U.S.D.A., Washington 25, D. C.
[2] Front Royal, Virginia.

comparison of the previous calving dates of all cows which failed to calve with the previous calving dates of all cows which calved in both years, (3) the association between calving date of cows when these calvings occurred in successive calving seasons.

In calculating the percent of cows calving each 20-day period during the calving season which failed to calve the following year the time interval of January 1 to August 8 inclusive was used. A cow was considered to have calved during the following year if she gave birth to a calf during the regular calving season as determined by the previous breeding season.

The effect of date of calving on subsequent calving performance was estimated by comparing the previous calving date of all cows failing to calve with the previous calving date for all cows that did calve. This comparison was made for each year for each breed. In assembling the data any cow which was with the bull less than 60 days following calving was not used for comparison in that year. This procedure largely eliminated from this comparison cows bred later than the end of the breeding season in the previous year and many cows which were brought in as bred cows.

The relationship between calving dates of cows when calvings occurred in successive calving seasons was determined by the method of linear regression and zero order correlation. The analysis was made by breeds on a within year basis since the average calving date was not constant in the years during which these data were collected.

Results

The fraction and percent of cows calving in each 20-day period which did not calve the following year is given in table 1. The fraction used to determine the percent had as a numerator the cows calving during the 20-day period which did not calve the following year and as a denominator the number of all cows which calved during the 20-day period. There was a definite relationship between the percent of cows not calving in the subsequent year and the time of calving in the previous year (when the breeding season was limited to a 90-day period). The correlation between percent of cows failing to calve and previous calving date (grouped in 20-day periods) was 0.95 with 10 degrees of freedom (statistically significant, $P<.01$). The regression of the percentage of cows failing to calve on previous calving date was 6.1% per 20-day period or 6.1% more cows not calving for each delay of 20 days in previous calving date. The effect of date of calving on calving percentage in the following year seemed to be associated with the overall calving percentage of the herd. Thus while the calving percentage of the Angus cows was larger than for either the Shorthorn or Hereford cows, the effect of calving date on percent of cows failing to calve was less apparent in the Angus than in either the Herefords or Shorthorns.

The previous calving date of cows failing to calve in a given year was

TABLE 1. EFFECT OF DATE OF CALVING ON THE NUMBER OF COWS FAILING TO CALVE THE FOLLOWING SEASON

Period	1	2	3	4	5	6	7	8	9	10	11
Date of calving	1 to 20	21 to 40	41 to 60	61 to 80	81 to 100	101 to 120	121 to 140	141 to 160	161 to 180	181 to 200	201 to 220
Fraction of cows not calving in next season											
Angus	1/17	11/121	7/123	6/65	7/43	7/31	2/9	0/2	1/1	1/1	4/5
Hereford	1/16	8/81	15/106	15/76	15/67	14/53	16/39	5/20	5/14	3/5	0/1
Shorthorn	3/39	11/118	25/135	24/111	27/78	15/38	5/15	3/7	3/3	—	—
All cattle	5/72	30/320	47/364	45/252	49/188	36/122	23/63	8/29	9/18	4/6	4/6
Percentage of cows not calving in next season											
Angus	5.9	9.1	5.7	9.2	16.3	22.6	22.2	0.0	100.0	100.0	80.0
Hereford	6.3	9.9	14.2	19.7	22.4	26.4	41.0	25.0	35.7	60.0	00.0
Shorthorn	7.7	9.3	18.5	21.6	34.6	39.5	33.3	42.8	100.0	—	—
All cattle	6.9	9.4	12.9	17.9	26.1	29.5	36.5	27.6	50.0	66.7	66.7

TABLE 2. AVERAGE CALVING DATE OF COWS DIVIDED INTO TWO GROUPS ON BASIS OF CALVING PERFORMANCE IN THE SUBSEQUENT YEAR

Calving season	1950	1951	1952	1953	1954	1955	1956	Weighted average
Group A—Cows which calved the next year								
Angus	90.5(14)[a]	70.1(33)	57.2(43)	73.3(38)	61.9(60)	47.4(91)	44.5(109)	55.9(388)
Hereford	118.3(27)	107.8(32)	71.0(44)	68.9(67)	65.4(71)	55.2(58)	53.2(96)	69.2(395)
Shorthorn	83.2(29)	77.5(36)	73.0(56)	69.4(53)	49.8(77)	40.4(97)	41.4(99)	55.5(447)
Group B—Cows which did not calve the next year								
Angus	116.7(3)	75.8(4)	97.5(2)	73.3(6)	74.3(7)	48.0(8)	51.1(16)	65.4(46)
Hereford	137.3(3)	117.1(15)	96.1(14)	84.1(14)	78.4(24)	89.6(5)	56.7(15)	87.4(90)
Shorthorn	81.3(15)	82.7(7)	77.4(21)	82.7(18)	64.3(6)	69.6(27)	48.8(23)	70.9(117)
Group B—Group A Difference in calving date								
Angus	26.2	5.7	40.3	0.0	12.4	0.6	6.6	9.5
Hereford	19.0	9.3	25.1	15.2	13.0	34.4	3.5	18.2
Shorthorn	—1.9	5.2	4.4	13.3	14.5	29.2	7.4	15.4

[a] Numbers in parentheses indicate numbers of cows calving in each group.

consistently later than was the calving date of cows which did calve. These differences are indicated in table 2. The calving date in the previous year of cows which failed to calve was 9.5 days, 18.2 days, and 15.4 days later than the calving date of contemporary cows in the first year which calved in both seasons in the Angus, Hereford, and Shorthorn herds, respectively. The results in tables 1 and 2 are interrelated and the trend in one table could possibly be predicted from examination of results in the other table.

In addition to the effect of calving date on subsequent calf crop there was a significant positive correlation between calving date in successive years. Cows which calve late one year also tend to calve late in the next year. In the case of the Hereford and Shorthorn herds where the percent of cows not calving was somewhat higher than in the Angus herd, differences in calving date between the cows not calving and cows calving

TABLE 3. MEASURE OF THE ASSOCIATION BETWEEN TWO CALVING DATES OF A COW IN SUCCESSIVE CALVING SEASONS (WITHIN YEAR, WITHIN BREED ANALYSIS)

Breed groups	Angus	Hereford	Shorthorn	All breeds
Correlation between two dates	0.327	0.375	0.457	0.367
Regression of second date on first date	0.265	0.291	0.445	0.323
Number of pairs of calving dates used	388	392	442	1222

was much greater and more consistent. The relatively smaller number of cows not calving in the Angus herd made these averages somewhat more variable and perhaps less reliable as indications of the effect of late calving on the chance of calving in the following year than the averages of Hereford and Shorthorn cows.

The association between date of one calving and date of calving in the next season is given in table 3. These data indicated that cows which calve late one year tend to calve late the next year if they calve at all. Cows tended to calve from 0.27 to 0.45 days later for each day later they calved during the previous year, depending on the breed. The correlation between calving dates and the regression of calving date on previous calving date were statistically significant ($P<.01$) for each breed.

Discussion. The foregoing results indicated a definite relationship between calving performance and time of calving in the previous year. Cows which calved late one year tended to calve late or not to calve in the next year. This phenomenon was due in part to the length of intervals in the reproductive cycle which produce a lower limit to the time elapse between successive calvings. The average gestation lengths and standard deviations reported by Burris and Blunn (1952) for Angus, Hereford, and Shorthorn cattle were 281.7 ± 7.1, 286.1 ± 5.6, and 284.2 ± 6.0, respectively. The interval between parturition and first estrus in the Front Royal herds

exceeded 100 days for 6.6% of the cows and 80 days for 16.5% of the cows (Wiltbank *et al.*, 1956). The interval between post partum estrus and beginning of pregnancy is the other component of the reproductive cycle.

If the time interval between calvings is greater than 365 days the cow will tend to calve later each year. When the breeding season remains at a constant time and of limited duration the cows which habitually have over 365 days between calvings will eventually not become pregnant during the breeding season and will miss the next year. Cows which require less than 365 days between the initiation of successive gestations will calve earlier each year until they approach a limit imposed by the beginning of the breeding season. Cows which calve early tend to have a longer time with the bull after the first estrus following breeding and thus may have several opportunities to be bred if they fail to conceive on the first breeding.

The correlations between calving dates in successive years and the correlations between calving date and calving percentage in the following year indicated that selection of cows which calved early would result in an earlier calf crop with a higher percent of the cows calving in the following year. These calves would also be larger at a constant weaning date according to the work of Evan *et al.* (1955) who reported that calves at weaning in a Hereford herd were approximately one pound heavier for each additional day of age at weaning.

Summary

Calving records of Angus, Hereford, and Shorthorn cows at the Beef Cattle Research Station at Front Royal, Virginia, from 1950 to 1956 were analyzed to determine the effect of calving date on subsequent calving performance. Three determinations made from these data include: (1) Percent of cows calving each 20-day period during the calving season which failed to calve the following season, (2) comparison of the previous calving dates of all cows which failed to calve with the previous calving dates of all cows which calved in both years and, (3) the association between calving dates of cows when these calvings occurred in successive calving seasons.

The regression of percent of cows failing to calve on previous calving date was 6.1% per 20-day period or 6.1% fewer cows calving for each delay of 20 days in previous calving date. The calving date in the previous year of cows which failed to calve was 9.5 days, 18.2 days, and 15.4 days later than the calving date of contemporary cows in the first year which calved in both seasons in the Angus, Hereford, and Shorthorn herds, respectively. The correlation between successive calving dates was 0.33, 0.38, and 0.46 for the Angus, Hereford, and Shorthorn herds, respectively. These observations indicate that selection for early calving date would result in an increase in calving percentage and an earlier calving date in the next year when the breeding season is limited to approximately 90 days.

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SOME EFFECTS OF PROGESTERONE AND ESTRADIOL ON THE OVARIAN STRUCTURES AND ON THE GONADOTROPHIC POTENCY OF THE PITUITARY GLAND OF THE GILT [1]

W. C. Foote, D. P. Waldorf,[2] H. L. Self and L. E. Casida

University of Wisconsin, Madison

PROGESTERONE has been used to synchronize estrual cycles in groups of breeding females for such purposes as artificial insemination, embryo transplantation and the shortening of the breeding season (Dutt and Casida, 1948; O'Mary *et al.*, 1950, with the ewe; Ulberg, *et al.*, 1951a; Baker *et al.*, 1954, with the sow; Christian and Casida, 1948; Willet, 1950, with the cow).

The mechanism by which progesterone has its effect is not clear. Ulberg *et al.* (1951a and 1951b) and Dutt (1953) have suggested that the inhibitory effect of progesterone on ovulation might be due either to interference with the production of the luteinizing hormone or with its release from the pituitary gland. The subsequent ovulation, when progesterone therapy was terminated, would result in the first case from resumption of luteinizer production or in the second case from release of the stored LH, the amount in storage perhaps having been increased during treatment.

Various workers (Robinson and Nalbandov, 1951; Byrnes and Meyer, 1951; Kammlade *et al.*, 1952) have indicated that the amount of gonadotrophin in the pituitary is a measure of the rate at which it is being secreted. However, this interpretation is based on associations observed between gonadotrophin potency and growth of follicles or changes in estrogen level and thus may be more applicable to FSH than to LH. That LH, however, may be stored and then released by the proper stimulus is suggested by Fee and Parkes (1929) and Hill (1934).

Ulberg (1955) has presented some evidence that the synchrony of estrual cycles in a group of heifers may be increased by injection of estradiol at the end of an artificial luteal phase. Estrogen may be able to cancel out the effects of progesterone or it may act as a tripping agent in releasing LH; in either case, it would control the time of the next estrus to a greater extent. That estrogen can facilitate the release of LH has been shown by Hohlweg (1934), Lane (1935) and Fevold *et al.* (1936). It was further shown that this can take place in the presence of the inhibitory effect of progesterone by Kidder *et al.* (1955) where stilbestrol injected into swine during the luteal phase of their cycle caused the luteinization of follicles. The single injection of a rather high level of estrogen in the

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[2] Ralston-Purina Fellow; deceased October 29, 1957.

present study was to test more particularly the cancelling effects of estrogen on progesterone which might then permit LH to be released. The possibility of the estrogen acting as a "tripping" agent was not excluded, but, in as much as only one dosage could be studied with the number of animals that could be used, a large dosage was chosen to test the possible antagonistic action.

The purpose of this study was to test for storage of LH in the pituitary glands of progesterone treated gilts and for antagonistic effects of estrogen which might bring about LH release in the presence of progesterone.

TABLE 1. EFFECTS OF PROGESTERONE AND ESTRADIOL ON THE GONADOTROPHIC POTENCY OF THE PITUITARY GLAND AND ON THE OVARIAN STRUCTURES OF THE GILT

Treatment group no.	No. gilts	Days of experimental period[a] indicating injection and slaughter schedule			Pituitary test[b]		Gilt ovarian condition	
		Progesterone injected	Estradiol injected	Animals slaughtered	Chick testis wt. (mg.)	Chick comb wt. (mg.)	Follicles 3 mm. or more in dia.	No. corpora lutea
I	9	....	..	18	51	96	19.9	11.3
II	11	1–17	..	25	54	86	7.6	14.1[c]
III	10	1–17	..	18	64	134	36.8	0
IV	10	1–17	14	18	64	140	9.2	0
V	9	....	14	18	60	107	3.7	11.7

[a] The first day of the experimental period was the 12th day of the estrual cycle.

[b] Comb and testis weights were transformed to their common logarithms and adjusted to remove effects of body weight. The values in the table are the antilogs of the averages of these transformed and adjusted values.

[c] Average of seven gilts ovulating.

Materials and Methods

Forty-nine market gilts of mixed breeding and averaging 232 lb. in weight were divided into five experimental groups. The gilts were checked daily for heat with vasectomized boars and were started on experiment individually after they had completed a normal estrual cycle. A cycle was considered normal if the animal returned to heat within a period of 18 to 23 days. During the experimental period the gilts were checked twice daily for heat.

The experimental period began on the 12th day of an estrual cycle with all treatments being timed from that stage. The hormones used were progesterone administered daily in a dosage of 0.4 mg. per lb. of body weight and estradiol given as a single injection of 20 mg. Both hormones were injected subcutaneously in corn oil. The days of the experimental period on which injections were made and animals were slaughtered are shown in table 1.

At the time of slaughter the pituitary glands were collected and desiccated in acetone. The ovaries also were removed from the animals and

the number of corpora lutea and the number and size of the follicles were noted.

In testing for LH the comb weights of 5-day-old white Leghorn Cockerels (13 days of age at autopsy) were used. The assumptions were made that comb growth is an indirect result of the action of the luteinizing on the interstitial cells of the testis to produce androgen and that the changes in testis weight were a result of both FSH and LH (Brenneman, 1936; Nalbandov *et al.*, 1946; Nalbandov and Baum, 1948).

Twenty-five mg. of the desiccated and finely ground gland were administered, subcutaneously, as a saline suspension in eight, equal, daily injections; autopsy was 24 hours after the last injection. Weights of the comb and the testis were obtained at autopsy. A dose response curve was developed with whole pituitary glands from gilts of approximately the same weight as those used in this study. Dosages ranging from 10–60 mg. were studied and 25 mg. of the prepared gland injected under the above conditions gave comb weights and also testis weights just below the midpoint between the control and 60 mg. dosage.

The weights of the pituitary glands (the smallest approximately 30 mg.) limited the number of chicks per gland to one. Equal portions from the remaining tissue of the larger glands were pooled, two glands to a pool, to increase the number of chicks per group; the data were combined for purposes of analysis. Thirteen such pools were made and injected into additional chicks increasing the total number used to 62.

The comb and testis weights were not normally distributed and they were transformed into their common logarithm to make them conform more closely to a normal distribution. These values were further adjusted by covariance to remove the effect of chick body weight before the final analyses were run.

Results and Discussion

Ovulation was inhibited in those groups receiving progesterone and slaughtered at the end of the injection period (groups III and IV, table 1).

Differences in follicular development can be seen by comparing the average number of follicles, 3 mm. or larger in diameter, for each of the groups (table 1). There was an average of 36.8 follicles for group III which was slaughtered at the end of the progesterone treatment period as compared to 19.9 follicles for the gilts of group I which received no exogenous progesterone but were killed on about the 9th day of their estrual cycles. It seems logical that this significant difference reflects the longer period of time which had elapsed since the previous ovulation in the progesterone treated group thus allowing a greater number of small to medium sized follicles to develop. The growth of vesicular follicles to a sub-ovulatory stage during the prolonged cycles in this progesterone treated group might be considered as resulting from the FSH that was

being secreted whereas the failure of maturation and ovulation of these follicles might have resulted from the absence of LH.

The effect of estrogen on follicular development can be noted by comparing the numbers of follicles in the ovaries of the gilts in those groups receiving estrogen to similar groups not receiving estrogen (IV *vs.* III, 9.2 *vs.* 36.8 and V *vs.* I, 3.7 *vs.* 19.9, respectively). These differences were significant ($P<0.05$) and indicate that estrogen perhaps by its inhibition of FSH suppresses the growth of small follicles. The obvious failure of follicles to mature and ovulate in these estrogen-treated groups indicates that this hormone under the conditions of this study was not effective in releasing LH from the pituitary. This failure in swine appears superficially at least to differ from the results of Ulberg (1955) in cattle.

In group II, where slaughter was delayed until 8 days after the end of progesterone treatment, development of large follicles and ovulation occurred, indicating that the gonadotrophic hormone responsible for this phenomenon had been released.

Testis weights of the chicks injected with pituitary glands from gilts slaughtered at the end of their progesterone treatment period (III, 64 mg. and IV, 64 mg.) tended to be heavier than those in the other groups (I, 51 mg.; II, 54 mg.; and V, 60 mg.; table 1) but these differences were not significant and do not indicate definitely a change in the general gonadotrophic content of the pituitary (FSH interacting with LH).

Significant differences were shown among the comb weights and they are interpreted as indicating differences in pituitary content of the luteinizing hormone among the different treatment groups. The effect of progesterone on the LH potency of the pituitary can be seen by comparing groups I and III (96 mg. *vs.* 134 mg.). This difference ($P\cong0.08$) suggests that the action of progesterone is one of blocking the release of LH from the pituitary with some consequent accumulation. Further evidence of the effect of progesterone is seen when group III is compared to group II in which slaughter was delayed until 8 days after the end of progesterone treatment (134 mg. *vs.* 86 mg.; table 1). The lighter comb weights in group II ($P<0.05$) indicate a release of LH from the pituitary after the injection of progesterone was terminated. The lack of effect of estrogen on the luteinizer of the pituitary can be seen by comparing comb weights from those groups receiving estrogen to similar groups not receiving estrogen (IV *vs.* III, 140 mg. *vs.* 134 mg. and V *vs.* I, 107 mg. *vs.* 96 mg., respectively). The failure of these to differ indicates again as it did in the observation on the ovaries that estrogen administration did not lead to a release of LH from the pituitary. As pointed out above a wider range of dosages needs to be explored.

Further effects of progesterone can be noted by studying the ovarian response in group II. Seven of the 11 gilts in this group ovulated with an average of 14.1 ova. This can be compared to averages of 11.3 and 11.7 ova for groups I and V, respectively, where no progesterone was given

($P<0.05$). This difference is probably related to the phenomenon noted above of the development of a greater number of follicles in group III than in group I. It is presumed that a greater number of follicles developed also in group II and that an increased number of these had developed to a stage where they were sensitive to the luteinizing hormone and consequently matured and ovulated. Similar results were noted by Boyarsky (1947) in rabbits and Baker *et al.* (1954) in gilts.

The incidence of estrus in the different groups was very similar to that of ovulation. Progesterone treatment caused complete inhibition of estrus in all cases. In group II where slaughter was delayed until 8 days after the end of injection, 9 of the 11 gilts experienced heat. These nine animals include the seven that were previously reported to have ovulated and two others that were in a stage judged from the size of the follicles to be preparatory to ovulation.

Summary

Forty-nine gilts (average live weight 232 lb.) of mixed breeding were divided into five experimental groups. The experimental period began on the 12th day of an estrual cycle with all treatments being timed from that stage. Group I received no injections; groups II, III and IV received progesterone (0.4 mg. per lb. of body weight, subcutaneously) from day one through day 17 of the experiment; group IV also received 20 mg. estradiol and group V 20 mg. estradiol only on day 14. Group II was slaughtered on day 25 of the experiment while all other groups were killed on day 18.

The average number of follicles 3 mm. or larger on the ovaries of gilts in the five treatment groups was 19.9, 7.6, 36.8, 9.2, 3.7, respectively. Estradiol suppressed the development and maintenance of follicles beyond the 3 mm. stage. Prolongation of the cycle by progesterone allowed a significantly greater number of follicles to develop.

Pituitary glands were tested for gonadotrophic potency using five-day-old, White Leghorn cockerels. Chick testis weights for the five treatment groups were 51, 54, 64, 64 and 60 mg. and comb weights were 96, 86, 134, 140 and 107 mg. Significant differences were found only between comb weights. These differences between comb weights indicate that the action of progesterone in delaying heat and ovulation is to block the release of luteinizer from the pituitary rather than to suppress its production.

Withdrawing progesterone led to LH release but a single injection of 20 mg. estradiol in the presence of functional corpora lutea or of the exogenous progesterone did not.

Seven of 11 gilts in group II had ovulated at time of slaughter with an average of 14.1 ova. These differed ($P<0.05$) from the averages of groups I and V (11.3 and 11.7).

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THE RELATIONSHIP OF RATION AND INHERITANCE TO CERTAIN PRODUCTION AND CARCASS CHARACTERISTICS OF YEARLING STEERS [1]

T. C. CARTWRIGHT, O. D. BUTLER AND SYLVIA COVER [2]

Texas Agricultural Experiment Station

THE variation in market price of steers is associated with the market value of the carcasses which in turn are valued according to their presumed eating qualities. There is a dearth of reported data concerning the variables affecting eating quality of beef; therefore, breeders have no proven selection criteria for improving the eating quality of beef. To understand more fully the relationship of the eating quality of beef to ration, inheritance and other influences, it is necessary to understand the relationship of these variables to each other. The data reported by Cover *et al.* (1957) were suitable for giving evidence of some of these inter-relationships.

Materials and Methods

Available for study were 18 Hereford steers and 20 F_1 (Brahman sires x Hereford dams) steers born on the McGregor Station. They were treated similarly until after weaning when equal numbers of each breed or cross were self-fed a high-concentrate and a low-concentrate ration in the feed lot for a period consisting of 14 days adjustment, 140 days test and 13 to 16 days holding after the test before slaughter. The high-concentrate ration was a ground mixture of milo grain, hay and cottonseed meal, 60%, 30% and 10%, respectively; the low-concentrate ration contained these feeds in the proportion of 20%, 65% and 15%, respectively. Carcass and meat data were collected in the Meats Laboratory and Food Research Laboratory at the A. & M. College of Texas. These data were analyzed according to an analysis of variance outline presented previously by Cover *et al.* (1957).

Results and Discussion

Averages for live animal measurements and carcass weights are given in table 1 and for measurements of fat deposition in table 2; measurements pertaining to muscular development are presented in table 3.

Ration. Gain to 180 days of age and age at slaughter are independent of ration, and the significant differences observed cannot therefore be con-

[1] The animal and carcass work is cooperative with the U. S. Department of Agriculture and is a part of S-10 Southern Regional Beef Cattle Breeding Project.

[2] Associate Animal Husbandman and Geneticist, Substation 23, McGregor; Head, Department of Animal Husbandry, College Station; and Professor, Department of Home Economics, College Station, respectively.

TABLE 1. LIVE ANIMAL AND CARCASS MEASUREMENTS AVERAGED BY RATION AND BREEDING GROUP

Breeding group (B)	Hereford		Here. x Brah.		Statistical significance		
Ration concentrate level (R)	Low	High	Low	High	Breed	Ration	BXR
Birth weight, lb.	73.8	67.9	88.3	91.7	**		
Gain to 180 days age, lb.	287.1	271.6	312.2	285.4		**	
Gain on 140 day test, lb.	305.8	311.8	299.1	281.5			
Wt. per day of age, lb.	1.9	1.8	2.1	2.0			
Days age at slaughter	428.4	439.9	423.1	431.8		*	
Dressing percent	59.6	60.9	62.9	63.2	**		
Chilled carcass wt., lb.	435.1	457.9	499.8	502.7	*		
Chill. car. wt./day of age, lb.	1.0	1.0	1.2	1.2	**		
Feeder score[a]	4.8	4.6	4.2	4.1			
Slaughter score[a]	5.6	5.9	5.8	5.9			
Diff. between feeder and slaughter scores	0.8	1.2	1.6	1.7	**		

[a] Scores, averaged from three judges, range from 1 through 9 with 1 the least desirable, 9 the most desirable and 5 average.
* P less than .05.
** P less than .01.

sidered as due to ration. Allotments to each ration were made at random from each sire group after 180 days of age was reached. The differences may be important in the interpretation of values obtained subsequent to the 180-day weight as suggested by Cartwright and Warwick (1955). The measurements which are usually presumed to be affected by ration, gain on 140-day test, weight per day of age, slaughter score, dressing percentage, carcass grade and measures of carcass fat, do not show statistically significant differences due to this treatment. This lack of ration effect may be partially explained by the comparatively young age of the steers and the short feeding period as well as by the lower gain to 180 days of the higher concentrate group.

Hereford vs. Brahman x Hereford. The crossbred calves were approximately 20 lb. heavier at birth, 40 lb. heavier at 180 days of age and gained

TABLE 2. FAT DEPOSITION MEASUREMENTS AVERAGED BY RATION AND BREEDING GROUP

Breeding group (B)	Hereford		Here. x Brah.		Statistical significance		
Ration concentrate level (R)	Low	High	Low	High	Breed	Ration	BXR
Carcass grade[a]	7.9	6.3	8.1	7.6			
Separable fat 9–10–11 ribs, %	29.9	35.0	29.7	29.9			
Est. fat in carcass, %	27.5	31.5	27.3	27.4			
Est. marbling, ribeye, %	5.9	9.0	5.8	6.4			
Ether extract, ribeye, %	9.9	12.4	6.3	7.2	**		
Fat over 9–10–11 ribs, mm.	8.2	13.0	11.4	11.0			
Kidney fat, lb.	1.8	1.9	2.1	1.9			
Caul and ruffle fat, lb.	3.7	4.8	3.9	3.6	*		*
Kidney, caul and ruffle fat, lb.	5.5	6.6	6.0	5.5			

[a] High (1), av. (2) and low (3) prime; high (4), av. (5) and low (6) choice; high (7), av. (8) and low (9) good; and high (10), av. (11) and low (12) commercial.
* P less than .05.
** P less than .01.

20 lb. less on test, but only the birth weight differences were statistically significant. More extensive data from the same herd and including these data (Warwick and Cartwright, 1955) indicated significant sire differences for gain on test. Sire groups averages differed 41 lb. for the Herefords and 64 lb. for the crossbreds. If age differences were not influenced by breeds or sires, then chance significant differences exist among the sire groups within breeds. The maximum age difference was 38 days among the Herefords and 19 days among the crossbreds.

TABLE 3. MUSCULAR DEVELOPMENT MEASUREMENTS AVERAGED BY RATION AND BREEDING GROUP

Breeding group (B)	Hereford		Here. x Brah.		Statistical significance		
Ration concentrate level (R)	Low	High	Low	High	Breed	Ration	BXR
Area of eye muscle, sq. in.:							
Actual	8.2	8.6	9.0	9.4			
Per 100 lb. carcass	1.9	1.9	1.8	1.9			
Adj. to fat thick. and car. wt.	1.8	1.8	1.8	1.9			
Weight of eye muscle, oz.:							
Actual	23.7	24.9	28.0	30.1	*		
Per 100 lb. carcass	5.5	5.5	5.6	6.0			
Per day of age	.055	.056	.066	.070	*		
Separable lean, 9–10–11 ribs, %	52.0	49.8	53.4	52.6			
Separable lean/day age, lb.	0.9	0.9	1.1	1.0	*		
Estimated lean in carcass, %	57.6	55.8	58.7	58.1			
Estimated lean/day age, lb.	0.6	0.6	0.7	0.7	**		
Cushion round, lb.:							
Actual	34.3	35.4	40.3	40.5	**		
Per 100 lb. carcass	.079	.077	.081	.081			
Per day of age	.080	.080	.095	.094	**		
Ratio, lean-bone, 9–10–11 ribs	2.9	3.3	3.2	3.0			*

* P less than .05.
** P less than .01.

The crossbred steers gained more weight per day of age at slaughter and had a significantly higher dressing percent and chilled carcass weight per day of age, but sire differences were not significant. Chilled carcass weight per day of age probably more nearly measures true rate of beef production than any other criterion studied.

The crossbreds were scored lower as feeders and higher as slaughter cattle. The two scores are not entirely measures of the same characteristics and a difference in the two values may not be an interpretable unit itself, but it is an indicator of degree of change. It appears that the crossbreds, as indicated by a statistically significant larger difference in scores, either responded to the feed lot regime to a greater extent than the Herefords or that their longer legs, somewhat drooping rump and greater general ranginess were more acceptable to the judges as more flesh was added although they did not gain as much weight on test and did not add as much finish. The Herefords carried more finish than the crossbreds except for the measure of thickness of fat over 9–10–11 ribs and kidney fat, but ether extract was the only measure of fat for which the Herefords were signifi-

cantly higher. There was a breed difference in the amount of fat deposited at different locations. The only statistically significant differential response of breeds to ration was for caul and ruffle fat. The Herefords deposited considerably more on the higher grain ration whereas the Herefords on growing ration and crossbreds on both rations had values more nearly equal. This indicates that the Herefords utilize concentrates in producing fat to an advantage over roughages whereas crossbreds do not, even though the crossbreds possessed smaller GI tracts (Butler *et al.*, 1956).

Actual area of eye muscle was higher for the crossbreds because they were heavier, but smaller per 100 lb. carcass weight because eye muscle development is not directly proportional to weight increase. Adjusted according to the formula Ya=Y — 00053448 mm. fat thickness +.0045605 lb. wt. of side — 1.134780 reported by Butler (1957), the areas were very similar. Weight of eye muscle was similar to area except for per day of age, the crossbreds producing significantly heavier rib eyes. There were no significant sire differences for rib eye measurements. Knapp and Nordskog (1946), Knapp and Clark (1950) and Shelby *et al.* (1955) have reported heritability estimates of rib eye area of 69, 68 and 72%, respectively, after adjustment for weight of animal.

Measurements of separable lean in the 9–10–11 ribs and estimated lean in the carcass were closely parallel, being higher for the crossbreds in actual percent and on a per day of age basis, the latter being statistically significant. Differences between sires are statistically significant for separable lean per day of age, and an estimate of heritability of 5% was obtained. Such an estimate from small numbers and computed by the intra-sire correlation method may be considerably affected by sampling error. Cushion round, being a relatively lean cut, follows a pattern similar to those of the more direct measures of lean. The drooping rumps of the crossbreds, which may be stronger structurally, do not appear to be an undesirable conformation feature with respect to the percentage of cushion round.

Inter-relationships. Correlation coefficients were calculated between all of the production and carcass measurements. Since there were two rather distinct groups, Hereford and Brahman x Hereford crosses, intra-breeding group variances and covariances were used for computing separate breeding group and pooled coefficients. Pooled values that are considered of importance are given in table 4.

Birth weight was not significantly correlated with any of the subsequent pre-slaughter measurements, although significance was approached in the crossbred group with total gain on 140-day test, weight per day of age and carcass weights. Correlations involving birth weight and post slaughter measurements were generally low in all categories, three measures being statistically significant at the .05 level, although not given in table 4. These three measurements were in pounds per day of age for separable lean in 9–10–11 ribs, for estimated lean in carcass, and for

weight of cushion round for the two groups pooled. None of these relationships was significant in the Herefords and only the last two in the crossbreds when the groups were considered separately. Thus, the calves which

TABLE 4. COEFFICIENTS OF CORRELATION[a] BETWEEN PRODUCTION VARIABLES AND BETWEEN PRODUCTION AND CARCASS VARIABLES

Variables	Gain to 180 days	Total gain on 140-day test	Weight per day of age at end of test	Feeder score	Slaughter score
Total gain on 140-day test	0.16				
Weight per day age at end of test	.70	0.67			
Feeder score	.72	.06	0.49		
Slaughter score	.39	.36	.35	0.56	
Chilled carcass weight	.81	.51	.79	.59	0.56
Carcass grade	—.34	—.31	—.35	—.37	—.45
Sep. fat in 9–10–11 ribs, %	.59	.30	.52	.54	.59
Sep. fat in carcass, %	.59	.30	.52	.54	.59
Est.marbling in eye muscle, %	.30	.20	.36	.43	.53
Ether extract in trimmed ribeye,%	.00	—.07	—.04	.24	.34
Fat thickness over ribeye, mm.	.56	.26	.50	.59	.49
Kidney fat lb. per 100 lb. carcass	.36	.08	.26	.10	.11
Caul & ruff. fat per 100 lb. carcass	.22	.12	.28	.05	.20
Sum inter. fat lb. per lb. carcass	.30	.12	.31	.08	.19
Eye muscle area	.31	.39	.38	.17	.34
Eye musc. area per 100 lb. carcass	—.57	—.17	—.46	—.50	—.29
Eye muscle area, adjusted	—.09	.17	.02	—.17	.06
Eye muscle, oz.	.29	.28	.35	.24	.34
Eye musc. oz. per 100 lb. carcass	—.27	—.05	—.18	—.18	—.02
Eye muscle oz. per day of age	.16	.30	.35	.11	.21
Sep. lean 9–10–11 ribs, %	—.56	—.24	—.45	—.54	—.54
Sep. lean 9–10–11 ribs, lb. per day age	.29	.41	.57	.10	.06
Est. lean in carcass, %	—.56	—.24	—.45	—.54	—.54
Est. lean in carcass, lb. per day age	.49	.58	.81	.20	.14
Cushion round, lb.	.72	.55	.74	.54	.52
Cushion round, lb. per 100 lb. carcass	—.65	—.22	—.55	—.45	—.38
Cushion round lb. per day age	.54	.64	.84	.34	.31
Ratio, lean-bone 9–10–11 ribs	—.11	.15	.17	.01	.13

[a] Pooled correlation coefficients for both breeding groups. Values required for statistical significance are: .05 level, 0.33; .01 level, 0.42; and .001 level, 0.52.

were larger at birth tended to develop more lean meat. This conclusion is similar to that of Woodward *et al.* (1954) who found birth weight more highly correlated with subsequent growth than with carcass quality.

The correlation coefficients of gain to 180 days with weight per day of age and with chilled carcass weight are fairly high. Weight per day of age at end of test was influenced more by gain to 180 days of age than by gain on 140-day test. Stonaker (1956) suggested a revised selection index that

gives weaning weight four times as much weight as daily gain in the feed lot. Gain to 180 days was closely correlated with feeder score (.72) but the carryover to slaughter score and carcass grade was small, especially in the crossbreds. These correlation coefficients for the Herefords agree fairly closely with similar values reported by Woodward (1954). The generally less acceptable appearance of the crossbreds as slaughter animals tends to diminish after the animals are slaughtered and on the hook. The animals that were heavier at 180 days apparently carried more fat (percent of body weight) when they were slaughtered as estimated from the 9–10–11 rib cut. Marbling and ether extract of the eye muscle as well as caul and ruffle fat were not significantly correlated with this weight; whereas fat thickness over the ribeye muscle, especially in the crossbreds, tended to be greater as gain to 180 days increased. The eye muscle measurements were not significantly correlated with gain to 180 days except on a 100-lb. carcass weight basis.

Correlations with total gain on 140-day test revealed that feeder score was a poor predictor of gaining ability but somewhat better as a predictor of slaughter score. Carcass grade improved as gain on test increased but not significantly, indicating that the slow and fast gainers did not finish at greatly different rates. The other correlations involving gain on 140-day test are similar for the Herefords and the crossbreds and are not significant except for the ones involving measurements that are directly affected by total weight or gain, such as actual eye muscle area. For those characters reported by Woodward (1954), agreement is rather close.

Feeder score and slaughter score correlations were similar with all post slaughter characters. The heavier Herefords, but not crossbreds, tended to receive higher slaughter scores. Apparently, the crossbreds have a different growth curve and had not begun to level off and fatten to the same degree as the Herefords. Slaughter scores were only fair estimates of the carcass grades, but the scores and grades are definitely related. The slaughter score was a fair indication of the amount of fat that the animal carried in all of the measures obtained except those of the internal fat. The scores were useless as indicators of adjusted eye muscle area and cushion round. Since the larger animals tended to grade better, actual weight of the eye muscle and cushion round were correlated with score. Ratio of lean to bone was neither significantly nor highly correlated with any of the other variables studied.

Summary and Conclusions

The work reported here represents a further analysis of production and carcass data from 18 Hereford and 20 Brahman x Hereford steers. These steers were treated similarly until weaning when equal numbers of each breed or cross were fed high and low concentrate rations for approximately 5 months and subsequently slaughtered.

Differences in concentrate content of the ration had little effect on the growth or other characteristics of the steers except that those which received the higher concentrate ration, especially the Herefords, were slightly higher in most measures of fat, whereas muscular development was less affected.

The crossbred steers had slightly higher carcass weights per day of age due mainly to heavier birth weights and higher gains to 180 days of age as well as higher dressing percentage. The scores or grades of the crossbreds, generally considered of less desirable conformation, tended to improve more with age than those of the Herefords. The Herefords tended to deposit more fat at more locations, but the fat deposition pattern is apparently different for the crossbreds since they deposited more fat at some locations. Differences due to sires were not statistically significant except for age and separable lean in 9–10–11 ribs per day of age for which heritability was estimated to be approximately 5%.

A correlation study indicated that birth weight, gain to 180 days of age and gain in the feed lot are all indications of growth potential and that weaning weight has the most marked influence on final weight. Growth at any period is positively correlated with scores or grades taken subsequently. Size of rib eye, weight of cushion round and other cuts changed percentage wise with changes in fatness but at the same degree of finish were markedly influenced only by differences in weight of the steer. Apparently an increase in the daily gain is the main influence in increasing the lean of beef.

Selection for higher pre- and post-weaning gains in yearlings should improve carcass grade slightly. If the trend toward "meat type" cattle continues to an extent similar to that in swine, roughage rations and gaining ability should be emphasized more. Visual appraisals were not related to performance or carcass quality other than fat, indicating little usefulness of such values. However, present day standards, although increasingly dynamic, still place considerable sales value on visual appraisal of conformation.

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DILUTION AND STORAGE OF BOAR SEMEN

P. J. Dziuk [1]

Illinois Agricultural Experiment Station, Urbana

ONE of the major obstacles to widespread use of artificial insemination in swine has been the difficulty encountered in maintaining motility and fertility of boar semen following dilution and storage (Polge, 1956a). The most satisfactory diluents on the basis of preservation of motility seems to be those containing glucose or glycine and egg yolk or milk (Roy, 1955; Polge, 1956a; Du Mesnil du Buisson, 1956; Du Mesnil du Buisson and Dauzier, 1956). Studies by Ito *et al.* (1948) indicated that the optimum temperature for storage of undiluted semen was 15–20° C. For the more highly concentrated semen obtained by fractional collection, storage at 3–10° C. seemed optimum for preservation. Coronel and Masankay-Arenas (1954) found boar sperm were preserved longer if stored at "ice box" temperature as compared to room temperature. Du Mesnil du Buisson and Dauzier (1956), Roy (1955) and Polge (1956b) stored the diluted semen at 5–6° C.

The following study was undertaken to evaluate and to improve various methods of preserving and extending boar semen.

Experimental

Four hundred semen samples were collected from seven boars by means of an artificial vagina. Purebred Duroc, Hampshire, Yorkshire and crossbred Hampshire-Yorkshire boars ranging in age from 7 months to 4 years were used. Each boar was housed separately and hand-fed to maintain a relatively uniform degree of fatness. Collections were made from each boar every 72 hours. Four of the boars were on this collection schedule for 9 months; the other three were used for about 2 months. The boars were trained to mount a dummy sow substituted for a live sow after they had established a favorable association with the collection procedure and surroundings. All collections were made out-of-doors without protection from weather at temperatures ranging from −6 to 96° F. None of the boars refused to mount the dummy and serve the artificial vagina under this regime.

An artificial vagina of the type described by Polge (1956b) was used at first, but collections were not consistent because of difficulties in providing a vagina of optimum length and pressure for each individual. All semen collections reported in this paper were made with an artificial vagina

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slightly modified from the type described by Aamdal *et al.* (1958). In our procedure the insulated, polyethylene collection bottle was attached to the collection funnel only after the sperm-rich fraction began to appear in the ejaculate. Straining of semen through cheese-cloth to remove the gelatinous material as compared to decanting the fluid portion reduced motility of the sperm. Consequently, separation was made immediately after collection by decanting the fluid, sperm-containing portion from the collection bottle.

The raw semen was cooled to room temperature over a period of about an hour and was always diluted at room temperature. Semen stored at 7° C. was cooled from 24° C. to 7° C. at a rate less than 5° per hour, because preliminary studies indicated sperm motility was reduced after storage if semen was cooled at a faster rate. This is presumably due to cold shock damage. Evaluation of sperm motility after storage in several pilot trials indicated that raw semen or diluted semen could be stored satisfactorily at room temperature for less than 4 hours. Storing semen at room temperature for 8 hours or more prior to cooling reduced subsequent sperm motility.

Preliminary work on dilution rates indicated that dilution, prior to storage, of 1 part of semen with 1 part of diluter, by volume, yielded samples with lower sperm motility after storage, than samples containing 1 part of semen to 2, 4 or 8 parts of diluter. Just prior to examination of motility, diluter was added to the stored samples in such a way as to make sperm concentrations of all samples equal. All semen samples reported in this study were diluted at the rate of 1 part of semen to 2 parts of diluter.

Samples were stored in rubber-stoppered, glass test tubes in a water bath. Diluted semen was prepared for evaluation by warming a 3–4 ml. sample in a 37° C. water bath for 30–60 minutes. Motility estimates were made at 160 × magnification while a drop of the sample was on a glass slide maintained at 37° C. by a warm stage. A numerical value ranging from zero (no motility) to 6 (maximum motility) was given to each sample at the time of motility estimation. Raw semen samples were split into as many samples as there were diluters to be evaluated. The diluted samples were given a code number at the time of dilution to minimize possibility of bias at the time motility estimates were made. Each comparison of diluters was made within ejaculates and after identical storage periods of 1–10 days. Samples were usually examined at 48-hour intervals. All comparisons reported in this paper are between the motility scores of the standard diluter and the diluter being tested. Once an individual sample was rated as having no motility, it was discarded and no further comparisons were made with that sample. This tended to minimize the number of direct comparisons that could be made even though motility was maintained in the standard diluter for extended periods of time. Most trials were terminated after 10 days of storage even though sperm in many samples were still motile. Determinations of pH were made at room tem-

perature with a glass electrode pH meter. Osmotic pressure of each sample was determined with a Fiske osmometer.

Glucose was used as a constituent on the basis of the work of Kampschmidt *et al.* (1953) and Van Tienhoven *et al.* (1952) who found it to be a satisfactory metabolizable sugar for bull sperm. In developing diluters, it was assumed the most satisfactory pH for diluters might closely approximate the normal pH of boar semen. Sodium bicarbonate was added because of its effect in raising the pH of normally, slightly acid egg-yolk diluters. Kampschmidt *et al.* (1953) reported that sodium bicarbonate had low toxicity and good buffering capacity when used at low levels. Homogenized whole egg (Dunn and Bratton, 1950) was also used because of its effect in raising the pH of solutions of which it was a part.

On the basis of work of Polge (1956b), Coronel and Masankay-Arenas (1954), Du Mesnil du Buisson and Dauzier (1956) which indicated that the addition of antibiotics and sulfa drugs increased livability of stored semen samples, two levels of antibiotics at two different storage temperatures were tested for their effect on maintenance of sperm motility.

In development of diluters, components were added in amounts so that the final osmotic pressure was nearly isotonic. No effort was made to exhaust the numerous possibilities of constituents and combinations of constituents.

Results and Discussion

The constituents of the diluters and conditions under which dilution and storage of boar semen was attempted are listed in table 1. In preliminary trials sperm motility was considerably lower in all diluters containing sodium citrate at the concentration used, as compared to the standard diluter. Consequently it was not extensively used as a diluter component. Motility of sperm stored at 16° C. was often superior to that stored at 7° C. for the first 48 hours of storage, but motility was never superior after 48 hours. Quite often samples stored at 16° C. showed no motility after 72 hours. A large number of motility estimates taken daily, coincidentally with pH determinations, indicated that pH fluctuations did not significantly influence motility if the pH was higher than 6.7. The reduction in pH was attributed to bacterial action or sperm metabolism, since pH lower than 6.7 occurred more often after storage at 16° C. than 7° C.

A limited number of samples which contained antibiotics and which were stored at 16° C. had a higher motility rating than those not containing antibiotics (table 1), but they had a lower motility rating than did similar samples stored at 7° C. (table 2). The higher level of antibiotics in samples stored at 7° C. exerted a slightly detrimental effect on motility either through direct toxicity to the sperm or by unfavorably altering the diluter

TABLE 1. COMPOSITION, AND CHARACTERISTICS OF VARIOUS BOAR SEMEN DILUTERS AND THEIR COMPARATIVE VALUE IN MAINTAINING SPERM MOTILITY

Diluter number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Egg yolk, ml.	30		30	30	30	30	30	30		30						30		30		30	30				30	30
Homogenized whole egg, ml.		50							30		50						50		50			50	30	30		
Dry skim milk, gm.													9.5	9.0	4.5											
Glucose, gm.	3.0	3.0	5.0	3.0		5.0			3.0	3.0	3.0				1.5	3.0	3.0	3.0	3.0	1.5				2.0	2.0	
Sodium bicarbonate, gm.	.15					.15	.15	.15	.15	.15				.15		.15		.15					.10			.20
Glycine, gm.					2.0		1.5	2.0												1.0						
Sodium citrate, gm																					2.9	2.9	2.9	1.0	1.0	2.0
Penicillin, units $\times 10^5$																1	1	1	1							
Streptomycin, mg.																100	100	100	100							
Distilled water, ml.	70	50	70	70	70	70	70	70	70	70	50		100	100	100	70	50	70	50	70	70	50	70	70	70	70
Storage temperature, °C.	7	7	7	7	7	7	7	7	7	16	16	7	7	7	7	7	7	16	16	7	7	7	7	7	7	7
Average pH diluter—semen	7.2	7.5	6.7	6.8	6.8	7.3	7.3	7.2	7.7	6.3	6.8	7.7	7.2	7.2	7.1			7.0		6.9	7.0			7.2		
Number of samples in pH average	401	160	29	25	14	3	16	7	3	85	110	179	47	28	87			10		19	2			7		
Average osmotic pressure, milliosmols	287	302	351	288	342	351	325	410				309	260		267					343	391			290		
Number of samples in osmotic pressure average	17	3	1	14	7	2	2	1				21	1		4					3	2			2		
Sample superior to diluter 1,%		15	8	7	11	0	4	0	0	18	17	16	11	9	25	40	0	76	57							
Sample equal to diluter 1,%		49	17	52	17	27	24	0	62	35	29	11	25	21	35	60	100	12	29							
Samples inferior to diluter 1,%		36	75	41	72	73	72	100	38	47	54	74	63	70	40	0	0	12	14							
Number of direct motility comparison to diluter 1		136	36	44	53	11	25	4	8	79	72	57	63	33	101	5	3	8	7							

(table 2). The results of this study are in accord with the work of Du Mesnil du Buisson (1956).

Under the conditions of this study, maintenance of motility was the most satisfactory when semen was stored at 7° C. in a diluter consisting of 3 gm. glucose, 0.15 gm. sodium bicarbonate, 30 ml. egg yolk and 70 ml. of distilled water. The addition of 1000 units of penicillin and 1 mg. of streptomycin per ml. of diluter also seemed beneficial. Motility was maintained satisfactorily in diluters consisting of 50 ml. homogenized whole egg, 3 gm. glucose and 50 ml. of distilled water or 4.5 gm. dry skim milk,

TABLE 2. COMPARATIVE ABILITY OF A DILUTER WITH TWO DIFFERENT LEVELS OF ANTIBIOTICS TO MAINTAIN SPERM MOTILITY AT TWO DIFFERENT TEMPERATURES

Diluter[a]	Antibiotic per ml. of diluter		Storage temperature	Direct motility comparisons to diluter 1			
	Penicillin	Streptomycin		No. of comparisons	Superior	Equal	Inferior
	units	mg.	° C.		%	%	%
1	1000	1.0	7				
2	1000	1.0	16	38	5	0	95
3	1500	1.5	7	74	22	43	35
4	1500	1.5	16	47	6	19	75

[a] Basic constituents were 30 ml. egg yolk, 3 gm. glucose, 0.15 gm. sodium bicarbonate and 70 ml. of distilled water.

1.5 gm. glucose and 100 ml. of distilled water. The affect of the addition of antibiotics was not tested for these last two diluters.

Insemination studies are now under way to determine the fertilizing ability of sperm stored under conditions which were found to be optimal in this experiment.

Summary

Techniques for handling, diluting and storing boar semen are described. Several different diluters were compared in their ability to sustain sperm motility under conditions in which semen was diluted at the rate of 1 part of semen to 2 parts of diluter. On this basis the most satisfactory diluter consisted of 3 gm. glucose, 0.15 gm. sodium bicarbonate, 30 ml. egg yolk, and 70 ml. of distilled water plus 100,000 units of penicillin and 100 mg. of streptomycin. Maintenance of motility was superior in samples stored at 7° C. than in those stored at 16° C.

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FERTILITY OF BOAR SEMEN ARTIFICIALLY INSEMINATED FOLLOWING *IN VITRO* STORAGE [1]

P. J. Dziuk and G. Henshaw [2]

Illinois Agricultural Experiment Station, Urbana

THE value of artificial insemination in swine would be greatly enhanced if it were possible to store boar semen *in vitro* for several days. One of the critical points in preservation of boar semen, is the fact that fertilizing ability is reduced after storage for 24 hours or longer (Polge 1956a). Ito *et al.* (1948) observed that the percentage of sows conceiving and their litter size decreased markedly as the length of time between collection of semen and insemination increased. Polge (1956b) found a marked reduction in the percentage of sows becoming pregnant to semen stored for 24 hours or more but only a small reduction in litter size.

The purpose of this study was to determine if apparent improvements in semen diluters, as judged by their ability to maintain sperm motility, (Dziuk 1958) are also indicative of their ability to maintain fertility.

Experimental Procedure

Semen was collected daily from one or more purebred Duroc, Yorkshire, Hampshire, or Yorkshire-Hampshire crossbred boars. Each boar was ejaculated at 72-hour intervals. The semen was handled by previously described techniques (Dziuk 1958). When fresh undiluted semen was used, it was inseminated within 30 minutes after collection and at the rate of 50 ml. per sow or gilt. Except for very few cases, the diluted semen was inseminated at the rate of 100 ml. for both sows and gilts. Semen was stored in a diluter composed of 30 ml. egg yolk, 3 gm. glucose, 0.15 gm. sodium bicarbonate and 70 ml. of distilled water. No antibiotics were added to the semen inseminated fresh or stored less than 3 days. Semen stored for 3 days prior to insemination contained 1000 units of penicillin and 1000 mcg. of streptomycin per ml. of diluter. Semen was diluted at the rate of 1 part of semen to 2 parts of diluter. The diluted semen was stored at 7° C. in 400 ml. Erlenmeyer flasks closed by rubber stoppers. Stored semen was warmed to about room temperature before being inseminated. An average of 7,544 billion (range, 2,000 billion to 18,000 billion) sperm were inseminated at each service.

The insemination equipment consisted of a glass 50 ml. syringe, a connecting piece of polyethylene tubing and one of several types of insem-

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[2] Present address: Assistant County Agent, Cadiz, Kentucky.

inating pipettes. Some inseminations were made with a rubber hose ½ in. in diameter (Polge 1956c). Most of the inseminations were made with a disposable plastic pipette 16 in. long and 6 mm. in diameter, such as is used in routine cattle inseminations. Some inseminations were also made with this type of plastic pipette but modified so that the diameter was ½ in. over the first 2 in. of the end inserted into the vagina.

Sows and gilts were initially detected in estrus by exposing the herd, once a day, to a teaser boar. Those females which allowed him to mount were considered as being in heat. Subsequently, to save time and to avoid excessive use of the teaser boar, most females were checked for heat by observing willingness to stand when a hand was pressed on the rump and by the appearance of the vulva.

No females were served by a vasectomized boar, but insemination routinely required several minutes so that some cervical stimulation probably occurred. In some cases, females were inseminated while restricted to a house or a small holding pen constructed of movable hurdles, but usually no restraint was required and it was considered unwise to excite animals by forcible restraint.

The females used in these experiments were primarily surplus animals from the University breeding herd with different nutritional, reproductive, and genetic histories. However, 40 of the animals inseminated with semen stored for 3 days were gilts of uniform weight and history. There was no selection of females on the basis of potential reproductive efficiency, and all females which were artificially inseminated are included in these data. No attempt was made to determine either optimum numbers of sperm or optimum volume of diluted semen for each insemination. Animals were inseminated either the first day of heat only, or the first and second day. Animals which returned to heat at a later estrous period were reinseminated.

Results and Discussion

The number of animals bred and conceiving is summarized in table 1. By comparing the percentage of females conceiving to first service with the percentage conceiving to all services, it is evident that some selection of the most fertile animals was made by the first service in the groups inseminated with fresh semen and that stored for 1 day. This was not true for the group inseminated with semen stored for 3 days.

The higher conception rate resulting from semen stored for 3 days, as compared to semen stored for 2 days may be due to the addition of antibiotics to the former. Du Mesnil du Buisson and Dauzier (1956) and Polge (1956c) also found that antibiotics raised the conception rate. Number of fetuses and embryonal mortality in females inseminated with fresh and one-day-old semen fall within the range generally observed in pigs bred by natural services. The statistically significant difference ($P < .05$)

TABLE 1. CONCEPTION RATES AND EMBRYONAL MORTALITY FOLLOWING INSEMINATION OF BOAR SEMEN STORED FOR VARIOUS PERIODS OF TIME

Age of semen at insemination	Number of first services	Number pregnant to first service	Per cent pregnant to first service	Number of total services	Total number pregnant	Per cent pregnant of total services	Average number of corpora lutea per pregnant animal	Average number of fetuses or ova per pregnant animal	Per cent embryonal mortality or unfer-lized ova	Number of days from insemination to examination	
										Av.	Range
1/2 hours	74	47	64	100	56	56	14.9	10.2	27	47	26–87
1 day	50	33	66	76	43	55	17.0	10.0	41	53	20–106
2 days	11	1	9	18	1	5	14.0	5.0	64	51	51
3 days	16	8	50	34	19	56	14.6	8.1[a]	20[a]	3.4	2–5
3 days	40	12	30	50	16	32	11.5	5.1	56	32	19–106
Combination of both 3 day groups	56	20	36	84	35	42	13.2	6.7	49	16	2–106

[a] Under the conditions of this experiment most of the ova were recovered from the uterus so that 69% of total ova were recovered, based on the number of ovulation points. There were 8.1 fertilized ova recovered per animal containing any fertilized ova and 20% of the total recovered ova were unfertilized, cytolyzed, fragmenting or giving other evidence of abnormality.

in conception rate between those animals inseminated with semen stored for 3 days and killed 4 days after breeding and those animals inseminated with semen of the same age and examined 25 or more days after insemination is of particular interest. There was a statistically significant ($P<.05$) but unexplainable difference between the numbers of corpora lutea found in the groups inseminated with semen stored for 3 days but examined at two different times. The embryonal mortality rate for those females inseminated with semen stored for 3 days is also higher than in those inseminated with fresh semen or that stored for 1 day. The differences in conception rate and embryonal mortality indicate that sperm stored for 3 days before insemination produce zygotes much more susceptible to early mortality. Salisbury *et al.* (1952) describe this same phenonemon in cattle.

A constant volume of diluted semen was inseminated, but in many cases considerable loss of semen from the vagina occurred during or shortly after insemination. The volume of this loss was recorded in a sufficient number of cases to indicate that in this study there was no relationship between the volume of semen lost at insemination and conception rate or litter size. It may be possible to inseminate pigs successfully using less volume of semen and smaller numbers of sperm than is now considered essential.

A single insemination per estrous period (128 cases) gave a conception rate of 40%. Insemination on two consecutive days (104 cases) of a single estrous period caused 50% of the females to become pregnant. These results are in accord with the findings of Aamdal and Hogset (1957). No critical attempt was made to assess the fertility level of each boar, but examination of available data revealed that each boar settled females at approximately the same rate.

The relatively low (42%) conception rate for all females inseminated with semen stored for 3 days is probably too low to allow artificial insemination of semen stored for 3 days to be practicable. This conception rate is superior to that reported previously (Polge 1956c) of 5% or less. Coronel and Masankay-Arenas (1954) obtained seven young from one sow inseminated with semen stored for 5 days, but their data do not permit an evaluation of the comparative fertilizing ability of stored sperm. The conception rate found in this study for semen stored 1 day (55%) is relatively high, as compared to the results of workers who stored semen for one day but used slightly different diluters (Polge, 1956b; Ito *et al.*, 1948; Polge and Rowson, 1956). Aamdal and Hogset (1957) achieved a conception rate of 59.9% using semen stored for 18–30 hours.

If the conception rates reported in this study are real increases over previously reported rates, further improvements in diluters and handling technique should permit more widespread use of artificial insemination in pigs.

Summary

Sows and gilts, unselected for reproductive performance, were artificially inseminated with fresh, undiluted semen which had been stored at 7° C. in an egg yolk-glucose-sodium bicarbonate diluter for various lengths of time. The conception rates for fresh, undiluted semen, and semen stored for 1, 2 and 3 days were 56% and 55%, 5% and 42%, respectively. Number of fetuses and embryonal mortality was normal in those cases in which fresh and one-day-old semen was used. Early embryonal mortality increased when the semen inseminated had been stored for 3 days.

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MINERAL REQUIREMENT OF RUMEN MICROORGANISMS FOR CELLULOSE DIGESTION *IN VITRO* [1]

FARRIS HUBBERT, JR.,[2] EDMUND CHENG AND WISE BURROUGHS

Iowa Agricultural and Home Economics Experiment Station, Ames

DURING recent years attention has been given to qualitative and quantitative requirements of rumen microorganisms for organic nutrients. A popular and productive realm of research has been centered around the identification of organic factors responsible for high activity of certain natural materials as stimulators of *in vitro* and, in some cases, *in vivo* cellulose digestion. Only limited attention has been given to the inorganic nutrient requirements of the rumen microflora, although the ash of such materials as alfalfa and molasses has been found to stimulate both *in vitro* and *in vivo* cellulose digestion. The need for an investigation of the mineral constituents of media used in artificial rumen studies was recently suggested by Warner (1956) in an evaluation of the various *in vitro* techniques being used.

An evaluation of the requirements by a mixed suspension of rumen microorganisms for various minerals for maximum *in vitro* cellulose digestion was believed desirable from several standpoints. First, the basal medium used for testing feed materials for stimulation of cellulose digestion by rumen microorganisms must contain an optimum level of inorganic nutrients to rule out the possibility of an apparent stimulation being a result of correcting a deficiency of some inorganic substance. Secondly, a measure of the toxic level of various inorganic elements would be of value both for *in vitro* studies and for possible relation to conditions that could exist in the rumen of the animal itself. Also, since studies of the mineral requirements of rumen microorganisms are very limited, a comparison of the relative importance of the various minerals in the artificial rumen with respect to cellulose digestion could be valuable when extended to conditions that exist within the rumens of all ruminants.

The objectives of this work were to determine the optimum and toxic concentrations in the fermentation medium for sulfur, magnesium, calcium, manganese, copper, cobalt, iron and zinc. Limited studies were made of the influence of boron, phosphorus and the carbonate ion.

Experimental

A washed suspension of rumen microorganisms was prepared essentially as described by Cheng *et al.* (1955). Rumen contents were collected from steers fitted with a permanent ruminal fistula maintained on high

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[2] Present address: Squaw Butte-Harney Range and Livestock Experiment Station, Burns, Oregon.

corncob rations. The nutrient medium shown in table 1 was used in all mineral studies involving requirements. Any source of the particular element under study was removed from the nutrient medium to provide a medium that was as free of that element as possible. Contamination of the fermentation medium from trace amounts of minerals in the reagent grade chemicals used cannot be disregarded. However, it was believed that the washed suspension method of preparing the microorganisms for the fermentation period separated most of the water soluble nutrients from the bacteria before they were placed in the fermentation tubes.

TABLE 1. COMPOSITION OF NUTRIENT MEDIUM USED IN THE STUDY OF THE MINERAL REQUIREMENTS OF THE RUMEN MICROORGANISMS [a]

Constituent	Amount
	gm./liter
$Na_2HPO_4.7H_2O$	1.20
KH_2PO_4	0.60
$NaHCO_3$	3.50
KCl	4.00
NaCl	4.00
$MgSO_4$	0.15
$CuSO_4.5H_2O$	0.002
$MnSO_4.H_2O$	0.004
$ZnSO_4.7H_2O$	0.00008
$FeSO_4.7H_2O$	0.075
$CoCl_2$	0.002
Urea	2.0

[a] Diluted 1:1 with distilled water containing the washed bacteria when added to the fermentation tubes.

The requirement for sulfur was studied by replacing all of the sulfate salts in the medium with the corresponding chloride salts in order to provide an equivalent amount of the cation. The various levels of sulfur studied were added to the tubes as Na_2SO_4. Magnesium requirements were studied by replacing the $MgSO_4$ in the medium with Na_2SO_4 on a sulfate equivalent basis. Calcium was studied by adding $CaCl_2$ to the unaltered medium. The various trace element requirements were studied by removing the particular element of interest completely from the medium. Experimental levels were added to the fermentation tubes in the sulfate or chloride form.

Experimental treatments were applied in a completely randomized design with two fermentation tubes per treatment in the various studies. Variability was determined by analysis of variance. Duncan's New Range Test (Duncan, 1955) was used to determine where statistically significant differences existed between treatment means.

Results

Sulfur. Results of two experiments shown in table 2 indicated that a range in concentration of from 10 to 500 or 600 mcg. of sulfur per ml. of fermentation liquid were required for maximum *in vitro* cellulose digestion. A slight depression in cellulose digestion was noted when sulfur was present in a concentration of 1,000 mcg. per ml. with further depression noted at the 2,000 mcg. level. However, the microorganisms were quite tolerant to the high sulfur concentrations studied as shown by the level of cellulose

TABLE 2. SUMMARY OF THE INFLUENCE OF THE ADDITION OF SULFUR OR MAGNESIUM TO THE FERMENTATION MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Trial 1 [a]		Trial 2 [b]		Trial 3 [c]		Trial 4 [d]	
S added	Digested	S added	Digested	Mg added	Digested	Mg added	Digested
mcg./ml.	%	mcg./ml.	%	mcg./ml.	%	mcg./ml.	%
1	9.6	0	39.9	10	44.5 [*]	0	40.8 [*]
0	18.6	2000	48.2	0	44.8	10	45.6
1000	33.0 [*]	1000	75.4 [*]	320	46.2	40	46.0
200	36.2	10	76.8	15	47.0	15	57.8
10	36.4	200	79.2	5	50.8	5	59.9
350	37.0	600	79.5	80	54.3	30	60.7
100	37.2	20	80.0	20	55.6	20	62.4
150	37.2	100	80.7	40	58.4	80	63.3
20	37.4	400	82.2	160	59.6	60	65.0
250	38.3					70	65.2
300	38.4					50	66.0
400	39.2						
50	40.8						
450	41.0						
500	44.2						

[a] Standard error of the mean is 1.98.
[b] Standard error of the mean is 1.16.
[c] Standard error of the mean is 4.0.
[d] Standard error of the mean is 2.7.
[*] All means contained within the same bracket are not significantly different from each other at a probability level of 0.05 or less.

digestion that took place at the 1,000 or 2,000 mcg. levels. The addition of 10 mcg. of sulfur per ml. to the sulfur deficient medium resulted in a significant increase in cellulose digestion of 17.8% in the first trial, and 36.9% in the second trial. The addition of 1.0 mcg. of sulfur had no beneficial influence.

Lewis (1954) has shown that sulfate is reduced to sulfide in the rumen. Feeding 150 gm. of $Na_2So_4 \cdot 10H_2O$ to sheep resulted in 14.7 micromoles of sulfide per ml. of rumen liquor; it had no ill effects on the animal. Thus, it is apparent that the ruminant is quite tolerant of high levels of sulfur. The need of sulfur by rumen microorganisms for cellulose digestion has also been shown by Hunt *et al.* (1954).

Magnesium. The addition of from 20 to 80 mcg. of magnesium per ml. in the one trial, shown in table 2, and from 20 to 160 mcg. of magnesium in another trial appeared to increase cellulose digestion over that in the

medium containing no magnesium. A level of 1,000 mcg. of magnesium per ml. was found to be toxic as shown by complete suppression of cellulose digestion. The essential role of magnesium for maximum cellulose digestion was not as clear cut as was true for sulfur; however, levels of from 20 to 160 mcg. gave consistent increases of approximately 10 to 20% cellulose digestion.

TABLE 3. SUMMARY OF THE INFLUENCE OF THE ADDITION OF CALCIUM OR MANGANESE TO THE FERMENTATION MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Trial 5 [a]		Trial 6 [b]		Trial 7 [c]			Trial 8 [d]	
Ca added	Digested	Ca added	Digested	Mn added	Digested		Mn added	Digested
mcg./ml.	%	mcg./ml.	%	mcg./ml.	%		mcg./ml.	%
0	47.6	500	38.8	0.1	57.8		320.0	40.0
100	49.2	450	40.8	2.0	62.2	[e]	0.1	47.0
1000	53.4	400	48.8	128.0	62.5		160.0	48.8
10	54.6	5	48.8	0.5	63.8		0.0	48.8
		0	48.9	32.0	64.2		80.0	49.4
		350	51.5	64.0	65.0			
		0.5	54.0	8.0	65.2			
		150	58.0	0.0	65.4			
		50	58.2					
		300	58.5					
		100	59.0					
		250	59.7					
		200	61.1					

[a] Standard error of the mean is 1.4.
[b] Standard error of the mean is 3.0.
[c] Standard error of the mean is 1.2.
[d] Standard error of the mean is 1.7.
[e] All means included within the same bracket are not significantly different from each other at a probability level of 0.05 or less.

Calcium. The addition of from 50 to 300 mcg. of calcium per ml. of fermentation medium appeared to give a consistent increase of approximately 10% cellulose digestion as shown in table 3. Although these increases were not statistically significant at a probability level of 0.05 or less, such consistent increase makes it appear likely that the rumen microorganisms do have a requirement for calcium. Burroughs *et al.* (1951) included calcium among the minerals required by rumen flora.

Levels of 450 mcg. or more of calcium significantly depressed cellulose digestion 10% below the response noted within the optimum range. However, it was noted in another trial that the addition of 1,000 mcg. of calcium per ml. did not depress cellulose digestion, so it appears that rumen microorganisms exhibit considerable tolerance to excess levels in the fermentation medium.

Manganese. The addition of manganese to the manganese-deficient medium failed to increase cellulose digestion under the conditions studied (table 3). A level of 320 mcg. of manganese per ml. of fermentation medium depressed cellulose digestion.

Copper. The addition of copper to the copper-deficient medium failed to increase cellulose digestion as shown in table 4. Relatively low concentrations of copper were noted to be toxic in both trials conducted with this element. Failure to obtain uniform response in duplicate tubes containing either 1.5 or 2.0 mcg. of copper resulted in an abnormally high standard error for the mean. Thus, it was not possible to get a sensitive measure of significant differences between treatment means in the two trials. However, it was apparent that a level of 1.5 mcg. of copper per ml. of fermentation medium was depressing cellulose digestion and a level of 2.5 mcg. essentially stopped cellulose digestion. McNaught *et al.* (1950) reported that 10 p.p.m. of copper was the maximum level that could be present in rumen liquid when the criterion of rumen microorganism activity was the conversion of non-protein nitrogen to protein.

Cobalt. The essential role of cobalt in the rumen for the synthesis of vitamin B_{12} has been adequately demonstrated; however, evidence of the need of cobalt *per se* for the digestion of cellulose by a mixed culture of rumen microorganisms is not to be found. The results of three trials, summarized in table 4, indicated that the addition of cobalt to the deficient medium did not increase cellulose digestion. As little as 5 mcg. of cobalt per ml. of fermentation medium was found to depress cellulose digestion approximately 12% when compared with the amount digested in the cobalt-deficient medium. This is in agreement with Hall (1955). McNaught *et al.* (1950) found that 10 p.p.m. of cobalt would reduce *in vitro* conversion of non-protein nitrogen to protein by rumen microorganisms. Salsbury, *et al.* (1956) using a procedure in which the strained rumen liquor was the inoculum for *in vitro* fermentation, reported that a level of 12 p.p.m. of cobalt depressed cellulose digestion.

It is possible that trace amounts of cobalt present as a contaminant in the various materials used in the *in vitro* systems for the study of the cobalt requirements met the microorganism needs; however, it is quite apparent that the rumen microorganisms are very sensitive to increased amounts.

Iron. The addition of iron to the fermentation medium failed to increase cellulose digestion in two trials summarized in table 5. In fact, it was found that the medium containing no added iron supported slightly higher cellulose digestion in both trials. A concentration of 300 mcg. of iron per ml. of fermentation liquid was found to significantly depress cellulose digestion.

McNaught *et al.* (1950) found that a concentration of from 10 to 100 p.p.m. of iron had no influence on the conversion of non-protein nitrogen to protein; however, 1,000 p.p.m. sharply reduced the synthesis when centrifuged rumen liquor was used as the medium. When phenanthroline was used as a chelating agent in McNaught's studies, rumen liquor containing from 1 to 2 p.p.m. of iron supported good protein synthesis. Hall (1955) reported the optimum concentration of iron in the fermentation medium to be from 1 to 8 p.p.m.

TABLE 4. SUMMARY OF THE INFLUENCE OF THE ADDITION OF COPPER OR COBALT TO THE FERMENTATION MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Trial 9 [a]		Trial 10 [b]		Trial 11 [c]		Trial 12 [d]		Trial 13 [e]	
Cu added	Digested	Cu added	Digested	Co added	Digested	Co added	Digested	Co added	Digested
mcg./ml.	%	mcg./ml.	%	mcg./ml.	%	mcg/ml.	%	mcg/ml.	%
4.1	4.1 [f]	2.5	3.4 [f]	50.00	30.2	0.005	34.8	50.0	1.3
8.2	7.4	1.5	19.9	0.50	36.7	0.05	35.6	5.0	30.3 [f]
2.0	31.8	2.0	28.3	0.55	40.8	0.0	37.4	0.55	48.9
0.5	57.8	0.5	47.0	0.0	42.6	0.0005	38.6	0.05	52.4
1.0	60.8	0.5	49.2	0.005	48.6	0.5	39.6	0.5	52.9
0.5	66.8	0.0	50.0					0.005	53.8
0.0	69.0	0.25	50.3					0.0	58.1
		1.0	51.4						

[a] Standard error of the mean is 10.6.
[b] Standard error of the mean is 8.5.
[c] Standard error of the mean is 10.9.
[d] Standard error of the mean is 3.0.
[e] Standard error of the mean is 2.3.
[f] All means included within the same bracket are not significantly different from each other at a probability level of 0.05 or less.

Zinc and Boron. The addition of as little as 5 mcg. of zinc per ml. of fermentation liquid significantly depressed cellulose digestion below that obtained with no added zinc, as shown in table 5. No benefit was obtained from the addition of zinc to the medium. Hall (1955) found that 16 p.p.m. of zinc depressed *in vitro* cellulose digestion by a mixed suspension of rumen microorganisms.

TABLE 5. SUMMARY OF THE INFLUENCE OF THE ADDITION OF IRON, ZINC, OR BORON TO THE FERMENTATION MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Trial 14 [a]		Trial 15 [b]		Trial 16 [c]		Trial 17 [d]	
Fe added	Digested	Fe added	Digested	Zn added	Digested	B added	Digested
mcg./ml.	%	mcg./ml.	%	mcg./ml.	%	mcg./ml.	%
1	40.0	500	5.4	50	5.4	50	31.2
15	47.6	300	29.3	5	24.0	5	36.0
10	47.7	100	34.1 [e]	0.05	36.8 [e]	0.5	35.5
8	49.2	5	37.5	0.002	40.8	0.0	40.8
2	49.5	50	39.3	0.0	40.8		
100	51.2	15	40.8				
16	53.4	0	49.6				
64	54.6						
4	54.6						
32	55.2						
0	57.4						

[a] Standard error of the mean is 3.4.
[b] Standard error of the mean is 1.1.
[c] Standard error of the mean is 1.7.
[d] Standard error of the mean is 5.2.
[e] All means included within the same bracket are not significantly different from each other at a probability level of 0.05 or less.

Boron was of interest in these studies since it was among the first trace elements shown to be essential for plant growth, yet has never been shown to be essential for animal life. The fact that it is found in appreciable amounts in the ash of alfalfa suggested the possibility that it might be of some importance to rumen microorganisms. However, the results shown in table 5 indicated that the addition of from 0.5 to 50 mcg. of boron was of no benefit.

Excess Levels of Phosphorus or Bicarbonate. The addition of as much as 1,000 mcg. of phosphorus per milliliter of fermentation medium did not depress cellulose digestion. Anderson *et al.* (1956) reported that the addition of from 40 to 80 mcg. of available phosphorus per ml. of fermentation medium met the requirements for *in vitro* cellulose digestion by rumen microorganisms. It was found necessary to deplete the microorganisms of phosphorus by incubating them for 24 hours in a phosphorus-deficient medium before the addition of phosphorus would increase cellulose digestion.

The addition as $NaHCO_3$ of as much as 4,000 mcg. of HCO_3^- per ml. of fermentation medium had no influence on cellulose digestion.

Discussion

The development of a washed suspension technique, such as that reported by Cheng *et al.* (1955), for the evaluation of the nutrient requirements of a mixed suspension of rumen microorganisms, necessitates a careful consideration of the composition of the basal fermentation medium used. This is true because the medium must contain adequate levels of all known nutrients, if a response resulting from the addition of experimental materials is to be interpreted as being due to an unknown nutrient. Sulfur, magnesium, and calcium appear to be the inorganic nutrients most likely

TABLE 6. SUMMARY OF THE OPTIMUM AND TOXIC CONCENTRATIONS OF VARIOUS MINERALS FOR MAXIMUM *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Element	Form added	Optimum elemental concentration	Toxic elemental concentration
		mcg./ml.	mcg./ml.
S	Na_2SO_4	10–500	1000
Mg	$MgSO_4$	20–160	320
Ca	$CaCl_2$	50–300	450
Mn	$MnSO_4.H_2O$	0–160	320
Fe	$FeSO_4.7H_2O$	0– 50	300
Cu	$CuSO_4.5H_2O$	0– 1	1.5
Co	$CoCl_2.6H_2O$	0– 0.5	5
Zn	$ZnSO_4.7H_2O$	0– 0.05	5
B	$Na_2B_4O_7.10H_2O$	0– 0	0.5

to be deficient in an artificial fermentation medium, as shown in the work reported herein. The important roles of potassium and sodium are considered in another report (Hubbert *et al.*, 1958).

The range of concentrations of the minerals found to support maximum *in vitro* cellulose digestion are summarized in table 6. The concentrations of sulfur, magnesium, and calcium found to depress cellulose digestion were high enough that the chance of introducing toxic levels via added experimental materials seems quite remote. It has been customary to use either a simple buffer or a complex medium patterned after the composition of saliva in most *in vitro* studies of rumen microorganisms. Warner (1956) compared the influence of a fermentation medium based on the composition of saliva with media developed from the composition of rumen liquor and with a simple phosphate-sodium chloride buffer. The main changes in the composition of the medium patterned after rumen liquor was to increase the potassium concentration, reduce the sodium concentration, and increase the concentration of calcium, magnesium, and sulfur. Increased microorganism activity was reported with the use of the meduim modified to conform more closely to rumen liquor composition. The results of

Warner's work, conducted at the Rowett Research Institute in Scotland, are in accord with the results of this research.

Manganese and iron were not found to be essential for optimum rumen microflora activity with the technique used in this study. However, the toxic concentrations of these minerals were found to be high enough that there would appear to be little danger of introducing toxic concentrations via added materials.

Additions of copper, cobalt, zinc, and boron did not result in increased cellulose digestion. The toxic concentrations of these minerals were found to be low. Thus, the possibility that inclusion of these trace minerals in the basal fermentation medium may depress cellulose digestion should be considered, especially where a washed cell procedure is being used. It appears that slightly higher levels of cobalt can be tolerated when rumen liquor is used in the fermentation medium (Salsbury, *et al.*, 1956). In subsequent *in vivo* studies these workers suggest that even lower concentrations of cobalt in the rumen were sufficient to depress cellulose digestion. Under practical feeding conditions, therefore, it would seem wise to use discretion in supplementing the ruminant ration with copper, cobalt, zinc, or boron.

Summary

A washed suspension, artificial rumen technique was utilized in a series of studies to determine the optimum concentrations of sulfur, magnesium, calcium, copper, cobalt, iron, and zinc for *in vitro* cellulose digestion by rumen microorganisms. Consideration was also given to toxic levels of these elements. Limited studies were made of the influence of boron, phosphorus, and the carbonate ion. Of the minerals studied, sulfur, magnesium, and calcium were found to be the inorganic nutrients most likely to be deficient in a prepared fermentation medium. Additions of maganese, iron, copper, cobalt, zinc, and boron to the fermentation medium did not result in increased cellulose digestion. Extremely low lewels of copper, cobalt, zinc, and boron depressed cellulose digestion.

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EFFECTS OF DIETARY STILBESTROL UPON CERTAIN ENDOCRINE ORGANS IN LAMBS [1]

JOSEPH D. SHRODER [2] AND SAM L. HANSARD [3]

University of Tennessee—Atomic Energy Commission Agricultural Research Program [4]

THE favorable effects of diethylstilbestrol (stilbestrol) upon rate of gain and efficiency of feed utilization in sheep and cattle have been reported by many investigators. However, few attempts have been made to delineate the mechanism or site of action for this material. The differences in response between animal species to stilbestrol treatment have complicated interpretation of results and prevented comparison of data between laboratory animals and ruminants. Nevertheless a profound physiological effect of stilbestrol has been reported for many classes of animals. Increased retention of calcium, phosphorus and nitrogen (Whitehair *et al.*, 1953; Bell *et al.*, 1955), heavier adrenals and pituitary glands (Cahill *et al.*, 1956; Clegg and Cole, 1954) and increased growth rate (Clegg and Cole, 1954; Andrews *et al.*, 1954; Perry, *et al.*, 1955; Shroder and Hansard, 1958) have been attributed to the effects of this substance upon the ruminant. Research reports concerned with the effects of stilbestrol upon the endocrine system, however, have been limited. It is the purpose of this paper to present results of studies upon endocrine glands from lambs fed stilbestrol to help clarify its mode of action in ruminants.

Experimental

Selected endocrine glands from ten crossbred wether lambs were used in this experiment. The lambs were maintained on a growing ration for 75 days as described by Shroder and Hansard (1958). One group of five lambs received the basal ration throughout; the other group received the basal ration plus sufficient stilbestrol to provide a daily intake of 4 mg. per head. At sacrifice, pituitary and adrenal glands were removed from all animals. The organs from the lambs were immediately trimmed free of extraneous material, blotted dry, weighed on a precision balance, and frozen until further studies could be made.

[1] Data presented herein were taken from a dissertation presented by the senior author to the Graduate School, Oklahoma State University, in partial fulfillment of requirements for the degree of Doctor of Philosophy.

[2] Formerly Graduate Fellow, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee. Present Address: Department of Animal Husbandry, Iowa State College.

[3] Present Address: Department of Animal Industry, Louisiana State University.

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Each anterior pituitary was pressed between two glass plates and was dried over concentrated sulphuric acid in a vacuum desiccator. The dried material was ground to a fine powder with a mortar and pestle and all residue which would not reduce to a powder was removed. The powder from all animals within each experimental group was pooled and stored in a desiccator for biological assay. Weighed portions of the pituitary powder from each group were then suspended as needed in a known volume of physiological saline for injection.

For assay of growth hormone, gonadotropic hormones and adrenocorticotropic hormone, 49 hypophysectomized, immature female rats weighing approximately 50 gm. were used 14 days following hypophysectomy. They were allotted at random into six groups and maintained during the experimental period on a commercial rat ration supplemented with dog food, fresh oranges, fresh milk, moist bread, and fresh potatoes. Thirteen rats were placed in each of two groups to receive injections of pituitary powder; six rats were placed in each of three groups representing three levels of purified growth hormone,[1] and five rats were retained as controls. Each treatment was replicated by dividing the animals receiving the treatment into two groups. Within the pituitary groups, each rat received 500 micrograms of pituitary powder intraperitoneally daily for 5 days. Those animals injected with purified growth hormone received 20, 100 or 300 mcg. of this material daily for 5 days and the controls were injected daily with sterile normal saline during the period. All surviving rats were sacrificed 18 to 24 hours after the last injection. The response to the growth hormone was measured using as a criterion the increase in width of the epiphyseal cartilage of the tibia as described by Greenspan *et al.* (1949). Gonadotropic hormone levels were measured using procedures described by Evans *et al.* (1940) and Heller *et al.* (1938), and adrenocorticotropic assay was accomplished by the method of Simpson *et al.* (1943).

For thyrotropic hormone assays, the procedure used was essentially that described by Bergman and Turner (1939). In addition, thyroidal activity was determined by measurement of radioiodine uptake as described by Rawson and McArthur (1947). Forty-eight day-old White Leghorn cockerels were divided at random into three groups, two pens per group, with eight chicks in each pen. The chicks in each pen received daily intraperitoneal injections of 300 mcg. of the appropriate pituitary powder for 5 days, and those in the third group received injections of sterile saline. All chicks were sacrificed the day after the last injection.

Analyses were performed to determine adrenal cholesterol levels in the control and treated lambs as a measure of adrenal function. The positive relationship existing between adrenal function and adrenal cholesterol has been pointed out by Sayers and Sayers (1948). Several small cross sections cut from each pair of adrenals were macerated with sterile sand,

[1] "Somar" generously provided by Armour and Company.

extracted with repeated washings of glacial acetic acid, and analyzed for total cholesterol by the method of Zlatkis *et al.* (1953).

Results and Discussion

The effects of dietary stilbestrol upon pituitary and adrenal glands from control and treated lambs are shown in table 1. Pituitary weights for the control and stilbestrol-fed lambs were significantly different ($P<.05$). Brody (1945) has cited evidence of a definite positive logarithmic relationship between body weight and pituitary weight, and when pituitary weights in this study were expressed as grams per hundred pounds of body weight,

TABLE 1. EFFECTS OF DIETARY STILBESTROL UPON PITUITARY AND ADRENAL GLANDS OF LAMBS

Item	Basal	Stilbestrol
Number of animals	5	5
Av. pituitary weight, gm.[a]	0.61(.04)[b]	0.81(.04)[b]
Av. pituitary weight, gm./cwt.	0.56(.04)	0.71(.04)
Av. % moisture of pituitary	86.40(.74)	86.10(.19)
Av. adrenal weight, gm.	2.84(.09)	3.20(.21)
Av. adrenal weight, gm./cwt.	2.62(.10)	2.80(.18)
Adrenal cholesterol, mg./gm.$\times 10^2$	7.10(.31)	8.10(.23)

[a] Anterior lobe only.
[b] Mean $\pm$ standard error of the mean in parentheses.

they were significantly higher for the treated lambs ($P<.05$). These data are in general agreement with similar data presented by Clegg and Cole (1954), Cahill *et al.* (1956); and Struempler (1957).

The percentage moisture values reveal that the increase in pituitary weight was not due to a large influx of water. Rather the increase in size might have been due to hypertrophy and/or hyperplasia, either of which would tend to indicate increased activity on the part of the organ.

Fresh adrenal weights, or these values converted to grams per hundred pounds of body weight, were not significantly affected by treatment. Christian (1953) has reported a definite logarithmic relationship between body weight and adrenal size, and since the stilbestrol-fed animals outgained their controls (Shroder and Hansard, 1958), it is apparent that adrenal size was not singularly affected by the dietary treatment.

Adrenal cholesterol content, used as a measure of adrenal cortical activity, was significantly less for the control lambs than for those receiving stilbestrol ($P<.05$). Apparently the adrenals of the stilbestrol-fed lambs were stimulated to a rate of secretion which was above normal. Supranormal levels of adrenal activity as evidenced in the treated lambs, however, are not consistent with the increased retention of nitrogen, indicative of protein anabolism, attributed to stilbestrol by many workers using

TABLE 2. GROWTH HORMONE ASSAY USING HYPOPHYSECTOMIZED IMMATURE FEMALE RATS

Treatment	No. of rats	Total material injected per rat	Final wt. of rats	Weight increase	Width of epiphyseal cartilage
		mg.	gm.	gm.	microns
Lamb pituitary:					
Basal	10	2.5	55.5	3.1	111.7(2.8) [a]
Stilbestrol	7	2.5	57.4	4.3	126.4(5.2)
Growth hormone	5	0.1	57.0	5.2	119.6(6.6)
Growth hormone	5	0.5	59.8	5.9	133.9(4.0)
Growth hormone	5	1.5	60.6	6.2	181.6(25.7)
Controls	4	Saline	52.2	0.7	76.9(3.4)

[a] Mean ± standard error of the mean in parentheses.

sheep and cattle (Whitehair *et al.*, 1953; Jordan, 1953; Clegg, 1952; and Clegg and Cole, 1954). Certainly the increased secretion of cortical hormones, if such occurs, is difficult to reconcile with the protein anabolic activity ascribed to stilbestrol. Sayers and Sayers (1948) have pointed out, however, that with a gradual change in environment, such as the stress of pregnancy and other causes, cholesterol level of the adrenals may drop initially, then gradually increase to a level higher than normal. Randall and Graubard (1940) likewise noted that in the later stages of pregnancy in rabbits, hypertrophy of the adrenal gland was accompanied by an increase in the absolute amounts of cholesterol. The increased estrogen level of pregnancy might possibly be compared with the continued administration of stilbestrol to explain the observed increase in adrenal cholesterol. If the increased adrenal cholesterol is a valid measure of adrenal activity, the data would indicate that the adrenal may be involved somehow in the response of lambs to stilbestrol. Results of pituitary assays for the growth hormone, adrenocorticotropic hormone, gonadotropic hormones, and for thyrotropic hormone are presented in tables 2, 3, 4 and 5.

TABLE 3. ADRENOCORTICOTROPIC HORMONE ASSAY USING HYPOPHYSECTOMIZED IMMATURE FEMALE RATS

Treatment	No. of rats	Total material injected per rat	Final wt. of rats	Weight of adrenals	
		mg.	gm.	mg.	mg./100 gm.
Lamb pituitary:					
Basal	9	2.5	55.5	6.9	12.2(2.5) [a]
Stilbestrol	7	2.5	57.4	7.2	12.7(4.8)
Hypophysectomized control	4	Saline	52.2	5.3	10.2(2.6)

[a] Mean ± standard error of the mean in parentheses.

TABLE 4. GONADOTROPIC HORMONE ASSAY USING HYPOPHYSECTOMIZED IMMATURE FEMALE RATS

Treatment	No. of rats	Total material injected per rat	Final wt. of rats	Weight of ovaries		Weight of uteri	
		mg.	gm.	mg.	mg./100 gm.	mg.	mg./100 gm.
Lamb pituitary:							
Basal	9	2.5	55.5	5.5	9.6(1.2) [a]	49.4	87.8(7.8) [a]
Stilbestrol	7	2.5	57.4	5.6	10.0(1.3)	29.6	51.2(3.7)
Hypophysectomized control	4	Saline	52.2	2.0	3.7(1.0)	11.0	21.2(1.8)

[a] Mean ± standard error of the mean in parentheses.

The growth hormone assay data (table 2) were expressed in terms of width of the epiphyseal cartilage of the tibias of hypophysectomized rats. The difference between the control rats and those receiving pituitary powder was highly significant ($P<.01$). Assay for growth hormone content of these pituitaries indicated a significant difference between the control and treated group ($P<.05$).

From data presented by Shroder and Hansard (1958), it was concluded that stilbestrol feeding increased growth in lambs. The assay data, if correlated with the increased growth of the animals from which the glands were taken, indicate that, per gram of pituitary tissue, there was increased production of growth hormone. However, there is no evidence to indicate that the entire growth response was due to increased growth hormone secretion.

Data from the adrenocorticotropic hormone assays appear in table 3. These data are in agreement with reports of Clegg and Cole (1954) and indicate that stilbestrol feeding has little effect upon ACTH secretion or storage in the pituitary gland. However, since the glands from stilbestrol-

TABLE 5. THYROTROPIC HORMONE ASSAY USING DAY-OLD CHICKS

Treatment	No. of chicks	Total material injected per chick	Final wt. of chicks	Thyroid wts.		I-131 uptake [a]
		mg.	gm.	mg.	mg./100 gm.	%
Lamb pituitary:						
Basal	13	1.5	53.1	4.6	8.9(0.9) [b]	7.78(1.0) [b]
Stilbestrol	15	1.5	51.5	4.9	9.8(0.7)	7.09(1.0)
Control	12	Saline	48.3	3.9	8.0(0.8)	3.69(1.0)

[a] Percent of the administered dose retained by the thyroids.
[b] Mean ± standard error of the mean in parentheses.

fed animals were larger, there was probably more total ACTH present, but this higher level was reflected only in an increased gland size which was consistent with the difference in adrenal size between the basal and treated animals.

The results of the gonadotropic hormone assay for the lamb pituitaries are presented in table 4. A comparison between the ovarian weights of the hypophysectomized control rats and those receiving pituitary powder indicated highly significant differences ($P<.01$). The values for uterine weights in the hypophysectomized controls were significantly lower than those within the lamb pituitary group ($P<.01$). As contrasted to ovarian weights, this difference in uterine weights within the lamb group was highly significant ($P<.01$).

The action of stilbestrol upon gonadotropic potency of the assayed pituitaries produced expected results; when the level of circulating estrogen increased as a result of stilbestrol administration pituitary production of gonadotropins decreased. It is highly questionable, however, that alteration of pituitary gonadotropin levels would be involved in the observed growth response from stilbestrol. Although it is not possible to correlate pituitary gonadotropin content with the growth response from stilbestrol, these data do demonstrate that stilbestrol has a systemic effect in lambs.

Thyrotropic hormone assays with day-old chicks were conducted using both increase in thyroid weight and percentage uptake of intraperitoneally administered iodine-131 by the thyroid as criteria (table 5). Differences in thyroid weights between the chicks receiving injections of saline or pituitary powder and between the control and treated pituitary groups were not significant.

The difference between the thyroidal iodine uptake of the saline-injected group and those receiving pituitary powder was significant ($P<.05$). The differences between groups receiving pituitary powder, however, were not significant, thereby giving no evidence of stilbestrol feeding affecting the thyrotropin content of the pituitary gland.

Summary

Investigations were conducted to study the interrelationship between dietary stilbestrol and certain endocrine organs and their products using growing wether lambs. Stilbestrol feeding significantly increased pituitary weights, and appreciably increased adrenal weights. When these weights were correlated with body weight, the stilbestrol-fed lamb pituitaries were still significantly larger than their controls. When adrenal weights were correlated with body weight, no differences were observed. Adrenal cholesterol levels, interpreted as being indicative of adrenal function, were significantly higher in the stilbestrol-fed lambs. Stilbestrol feeding resulted in a significant increase in pituitary growth hormone. Gonadotropin levels

were significantly reduced as a result of estrogen feeding. Hormone administration, however, apparently had little influence upon pituitary levels of ACTH and thyrotropin.

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THE INFLUENCE OF POTASSIUM, SODIUM, RUBIDIUM, LITHIUM AND CESIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS WITH OBSERVATIONS UPON SODIUM AND POTASSIUM INFLUENCES IN LAMB FATTENING RATIONS[1]

FARRIS HUBBERT, JR.,[2] EDMUND CHENG AND WISE BURROUGHS

Iowa Agriculture and Home Economics Experiment Station, Ames

THE important role of inorganic nutrients in stimulating metabolic activities of rumen microorganisms and similarly animal performance is well recognized. The digestibility by steers of a high corncob ration was increased by the presence of inorganic nutrients found in alfalfa (Burroughs *et al.*, 1950). Swift *et al.* (1951) reported that more feed energy was made available to lambs on a high corncob ration by the addition of alfalfa ash. Tillman *et al.* (1954) found that neither the addition of a complete mineral mixture nor alfalfa ash increased the digestibility of a prairie hay basal ration by sheep. However, Tillman, Sirny and MacVicar (1954) subsequently reported that the addition of 28 gm. of alfalfa ash to a lamb ration containing 60% cottonseed hulls improved the apparent digestibility of each ration constituent.

Shrenk and Silker (1950), in a study of the mineral composition of dehydrated alfalfa found potassium to be the most abundant mineral, averaging 2.60%. Reports on the quantitative requirements of rumen microorganisms for potassium are limited. Burroughs *et al.* (1951) included potassium among the minerals required by rumen microorganisms. The concentrations of soluble elements in rumen liquor reported by Phillipson (1953) showed potassium to range from 99 to 175 mg. per 100 ml. of rumen liquor. Reports of antagonistic and sparing relationships between sodium and potassium are to be found with chicks (Burns *et al.*, 1953), rats (Meyer *et al.*, 1950), and lactic acid bacteria (MacLeod and Snell, 1948).

The objectives of this work were to establish the requirements of a mixed suspension of rumen microorganisms for sodium, potassium, rubidium, lithium, and cesium for maximum *in vitro* cellulose digestion, and to study the influence of different levels of sodium and potassium added to a low-ash lamb fattening ration.

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[2] Present address: Squaw Butte-Harney Range and Livestock Experiment Station, Burns, Oregon.

Experimental

Procedures followed in preparing mixed suspensions of rumen microorganisms for 24-hour *in vitro* fermentation studies were essentially the same as those described by Cheng *et al.* (1955). Rumen contents were collected from steers fitted with rumen fistula and maintained on high roughage rations. Essentially sodium-free and potassium-free nutrient media, shown in table 1, were developed to study sodium and potassium requirements. Sodium and potassium additions were made as chlorides. Other alkali metals under study were also added as chlorides. The potassium-free

TABLE 1. COMPOSITION OF SODIUM-FREE AND POTASSIUM-FREE MEDIUM [a]

Constituent	Sodium-free medium	Potassium-free medium
	gm. per liter	gm. per liter
$NaH_2PO_4.7H_2O$	—	1.2
KH_2PO_4	1.2	—
$NaHCO_3$	—	3.5
$KHCO_3$	3.5	—
$MgSO_4$	0.6	0.6
KCl	4.0	—
NaCl	—	4.0
$CaCl_2$	0.55	0.55
Urea	2.0	2.0

[a] Diluted 1:1 with distilled water containing the washed bacteria when added to the fermentation tubes.

medium was modified to study the interrelationships of the various alkali metals by removing the sodium chloride to provide a low sodium, potassium-free medium. It should be pointed out that the potassium-free or sodium-free medium used here refers to the composition of the synthetic medium. It is possible that a trace of such ions may be present from the inoculum.

Generally, treatments were replicated once in a single 24-hour fermentation period in a completely randomized design. Although this procedure of replication usually resulted in a low standard error for the mean, it had the disadvantage of limiting the number of treatments that could be studied in a factorial experiment since approximately 50 fermentation tubes were the maximum number available for a single fermentation period. In certain studies involving more than 25 treatments only one tube per treatment was included during a single 24-hour fermentation period. A subsequent fermentation period including the same treatments was used for purpose of replication in a randomized block design. Analysis of variance in studying treatment effects and Duncan's New Multiple Range Test, as outlined by Duncan (1955) was used where appropriate to determine significant differences between treatment means.

A 70-day lamb fattening trial was conducted with 54 wether lambs having an initial average weight of 87 lb. The basal ration contained ground corn 48, ground corncobs 37, corn syrup 10, corn oil 2, urea 2, dicalcium phosphate 0.6, $MgSO_4$ 0.4, and limestone 0.2, with vitamins A and D added at the level of 1,000 and 100 I.U. respectively per lb. and diethylstilbestrol at the level of 600 mcg. per lb. It was fed to all lambs for 2 weeks prior to initiating the experiment, to accustom the animals to the ration. They were then eartagged, paint-branded, weighed, and randomly allotted to nine treatments (three levels each of sodium and potassium).

TABLE 2. INFLUENCE OF THE ADDITION OF SODIUM OR RUMEN LIQUOR TO A SODIUM-FREE NUTRIENT MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Sodium added	Rumen liquor added	Cellulose digestion [a]
mcg. per ml.	ml.	%
4000	0	16.3
2000	0	37.2 [b]
0	0	38.0
50	0	38.2
1000	0	38.6
500	0	48.2
0	1	62.2

[a] Standard error of the mean is 4.3.
[b] Means included within the same bracket are not significantly different from each other at a probability level of 0.05 or less.

The lambs were fed twice daily, for a 3-hour period, from individual self-feeders. They were held in groups of 10 or 11 in adjacent pens when not eating. Water but no salt was available in the holding pens. Weight and feed consumption data were taken at 2-week intervals. All weights were taken at 7 a.m. after a 12-hour shrink period in which the lambs were without feed and water.

The basal ration was formulated to meet the minimum known requirements of all essentials except sodium. The ingredients were chosen to provide a low-ash ration with special attention being given to maintaining as low a potassium content as possible and still have a ration that could supply adequate nutrients for some fattening. The ash, sodium, and potassium contents of the ration are summarized in table 6. Sodium and potassium were determined by the method of Hald (1947) using a flame photometer.

Results

Sodium Requirement. The addition of 50 to 4,000 mcg. of sodium per ml. of fermentation medium did not significantly increase *in vitro* cellulose digestion over a nutrient medium containing no added sodium (table 2). One ml. of rumen liquor was used as a positive control treatment since

TABLE 3. INFLUENCE OF THE ADDITION OF POTASSIUM OR RUMEN LIQUOR TO A POTASSIUM-FREE NUTRIENT MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Potassium added	Rumen liquor added	Cellulose digestion
mcg. per ml.	ml.	%
0	0	2.6
4000	0	27.9
500	0	34.8 [b]
2000	0	36.6
1000	0	37.8
0	1	51.8

[a] Standard error of the mean is 1.5.
[b] Means included within the bracket are not significantly different from one another at a probability level of 0.05 or less.

it is known that rumen liquor is a potent source of stimulatory factors for *in vitro* cellulose digestion. The apparent increase in cellulose digestion due to 500 mcg. of sodium was primarily due to the response in one fermentation tube, and was probably not a true treatment response. Further evidence that sodium is not essential for *in vitro* cellulose digestion by rumen microorganisms was obtained in another study shown in table 4.

Potassium Requirement. The presence of potassium in the fermentation medium was found to be essential for accelerated *in vitro* cellulose digestion. The addition of from 500 to 2,000 mcg. of potassium per ml. of fermentation medium was found to support a relatively high level of *in vitro* cellulose digestion as shown in table 3. The significant increase in cellulose digestion of approximately 15% over the optimum potassium, due to the addition of one ml. of rumen liquor per 20 ml. of potassium-free medium, emphasizes the potency of rumen liquor as a source of stimulatory factors.

TABLE 4. INFLUENCE OF ADDITIONS OF SODIUM AND POTASSIUM TO A LOW-SODIUM, POTASSIUM-FREE FERMENTATION MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS [a]

	Sodium added (mcg. per ml.)						
Potassium added	0	50	100	200	400	800	Av.
mcg. per ml.	%	%	%	%	%	%	%
0	4.8	1.4	2.1	1.6	1.9	3.2	2.5
25	6.3	11.0	5.3	3.0	1.8	5.6	5.5
50	24.5	16.7	16.8	20.2	7.9	5.4	15.7
100	22.0	28.8	24.2	25.0	28.6	32.8	26.2
200	29.2	25.2	27.4	30.0	30.4	37.8	30.0
400	33.8	30.6	31.8	30.4	34.8	40.3	33.6
Av.	20.1	18.0	17.9	18.3	17.7	21.4	

[a] Standard error of the mean is 4.6.

Sodium and Potassium Interrelationships. Interrelationships between varying concentrations of sodium and potassium in the fermentation medium are summarized in table 4. When 50 mcg. or less potassium per ml. of fermentation liquid were present, sodium was found either to have no influence or to depress cellulose digestion. However, when from 100 to

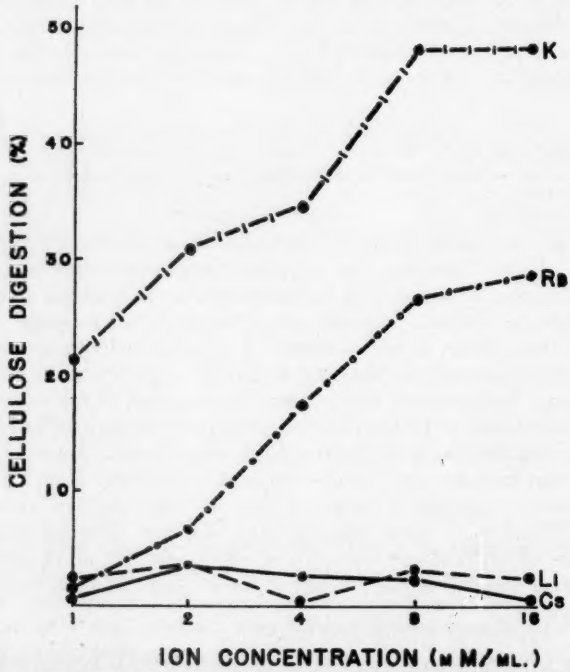

Figure 1. Influence on in vitro cellulose digestion of the addition of five levels of potassium, rubidium, lithium or cesium to a potassium-free fermentation medium.

400 mcg. of potassium per ml. were present the antagonistic influence of sodium disappeared and, in fact, the combination increased cellulose digestion over that resulting from the addition of potassium alone. Statistical analysis showed that there was a significant interaction of sodium and potassium.

That the chloride ion was not the limiting metabolite in these comparisons is evident in the results summarized in table 4, since sodium and potassium were added as the chloride. A recent study reported by Aines and Smith (1957) showed that sodium ions, but not chloride ions, were of therapeutic value to sodium chloride deficient cows.

Replacement Value of Rubidium, Lithium, and Cesium for Potassium. In view of the essential role found for potassium in the fermentation medium of rumen microorganisms, the work was extended to a study of the replacement value of other alkali metals for potassium. The results of the study in which 5 levels of potassium, rubidium, lithium or cesium were added to the potassium-free medium are shown in figure 1. The comparisons were made on a molar basis because of the wide range in atomic weights of the elements. Neither lithium nor cesium replaced the potassium requirement for *in vitro* cellulose digestion. However, rubidium was capable of replacing over 50% of the potassium requirement.

Rubidium has been reported by Glendening *et al.* (1956) to have a

TABLE 5. INFLUENCE OF ADDITIONS OF POTASSIUM AND RUBIDIUM TO A POTASSIUM-FREE FERMENTATION MEDIUM ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS [a]

Potassium added	Rubidium added (mcg. per ml.)					
	0	50	100	150	200	Av.
mcg. per ml.	%	%	%	%	%	%
0	2.8	7.8	15.6	35.6	42.0	20.8
50	27.2	45.0	48.8	52.0	50.9	44.8
100	50.0	57.6	56.0	60.5	58.4	56.5
150	64.6	58.8	61.4	62.0	56.2	60.6
200	64.2	60.0	59.5	57.8	60.0	60.3
Av.	41.7	45.8	48.2	53.6	53.5	

[a] Standard error of the mean is 4.2

slight and only temporary alleviating effect on potassium deficiency in rats. MacLeod and Snell (1948) found that rubidium would completely replace the potassium requirement for growth of *Lactobacillus casei* and *Streptococcus faecalis* bacteria, while for other lactic acid bacteria the potassium requirement could be replaced only partially or not at all with rubidium. In the same series of studies cesium would not replace potassium as an essential ion, and lithium was found to be more toxic than sodium when added to a low potassium medium.

A factorial study showing potassium and rubidium influences upon rumen microflora is summarized in table 5. It was found that the addition of 50 to 100 mcg. of rubidium in the presence of 50 mcg. of potassium per ml. of fermentation medium resulted in an increase in cellulose digestion slightly greater than would be expected from the additive effects of the two minerals. This positive interaction was not found at other concentrations in the study. The analysis of variance indicated that a significant interaction between potassium and rubidium levels did exist.

Lamb Fattening Trial. As shown in table 6 the addition of 0.96% potassium chloride or 1.65% sodium chloride to the low-ash basal ration was found to increase daily liveweight gains approximately 0.25 lb. per lamb per day and daily feed intake, 0.7 lb. This increase in liveweight gain paralleled the increase in cellulose digestion noted with potassium and sodium in the artificial rumen studies. This parallelism indicates that the ion effect on the rumen flora may be associated with animal performance. The combination of the two minerals at these levels resulted in a slightly higher rate of gain than either of the minerals alone. A summary of

TABLE 6. THE EFFECT OF SODIUM AND POTASSIUM ON AVERAGE DAILY GAIN AND DAILY FEED CONSUMPTION OF LAMBS DURING THE 70-DAY FEEDING PERIOD

Lot no.	Additions to basal ration		Composition of ration			Gain	Feed consumed
	KCl	NaCl	Ash	Na	K		
	%	%	%	%	%	lb.	lb.
1	0	0	2.47	0.01	0.39	0.03	2.09
2	0	1.65	4.12	0.66	0.39	0.25	2.75
3	0	3.33	5.80	1.32	0.39	0.23	2.56
4	0.96	0	3.43	0.01	0.89	0.28	2.81
5	0.96	1.65	5.08	0.66	0.89	0.30	2.80
6	0.96	3.33	6.76	1.32	0.89	0.06	1.89
7	1.92	0	4.39	0.01	1.40	0.20	2.39
8	1.92	1.65	6.04	0.66	1.40	0.21	2.59
9	1.92	3.33	7.72	1.32	1.40	0.19	2.52

cumulative gains indicated that the combination of sodium and potassium at the intermediate level resulted in a slight advantage in live weight gain throughout the entire experimental period.

In general, the addition of 1.92% potassium chloride or 3.33% sodium chloride alone or in various combinations, resulted in slightly less gain and feed consumption than smaller additions of the minerals. This is believed to indicate that with this type ration the higher levels of sodium and potassium were in excess of the requirements of the rumen microflora. That the type of ration would influence the tolerance of the rumen microorganisms to the level of sodium chloride, is indicated by the work of Meyer *et al.* (1955) in which as much as 12.8% sodium chloride was added to an 84% alfalfa ration without depressing gain or feed intake of fattening lambs. The very poor response to lot 6 was mainly due to extremely poor gains and feed consumption of two lambs. Examination of fecal samples indicated a marginal concentration of coccidia; however, scouring or symptoms of coccidiosis were not apparent.

Discussion

Although not previously reported, the finding that potassium and not sodium is required by rumen microorganisms for accelerated *in vitro* cellulose digestion is not surprising in view of similar work with other microorganisms. The apparent toxic influence of sodium when potassium was present in concentrations of 50 mcg. per ml. or less could be of major importance in *in vitro* studies where the basal medium is made up primarily of sodium salts. Fermentation media used in *in vitro* studies should be developed so that there is a concentration of at least 100 mcg. of potassium per ml. of medium, since the addition of sodium in any amount could depress cellulose digestion if the medium is deficient in potassium. When sodium and potassium are present at optimum level, it would appear unlikely that depressant amounts of either ion would be introduced by experimental materials being investigated because of the wide tolerance of rumen microflora for higher levels.

A consideration of the possible replacement value of the alkali metals rubidium, lithium, or cesium for potassium was of practical interest because of the reported presence of rubidium in alfalfa ash (Glendening *et al.*, 1955). The fact that rubidium was found capable of replacing potassium to some extent was not believed to be of importance from the standpoint of practical ruminant rations because of the low concentrations of rubidium found in natural feeds. However, it is of interest to note that rubidium was the only alkali metal found to be active in replacing potassium.

The antagonistic and additive effects of the ions observed in this work are most easily explained on the basis of competitive interference of one ion with the essential role of a somewhat similar ion as an activator in enzyme systems. This work suggests that a minimum concentration of 100 mcg. of potassium per ml. of fermentation medium meets the minimum requirements of rumen microflora for potassium with the fermentation procedure employed. If potassium is present in concentrations of 50 mcg. or less per ml., sodium may inhibit metabolic activity of the organisms by competing with potassium for the active sites of the potassium-specific enzyme systems. One can postulate further that the enzyme systems involved in the interrelationships observed in this work are likely located on the surface of rumen bacterial cells since media devoid of potassium or rubidium almost completely failed to support cellulose digestion. The washing procedure used in preparing the suspension of rumen organisms for the fermentation could not have materially influenced the intra-cellular concentration of potassium. Kitts (1953), working with a mixed suspension of rumen microorganisms, suggested that phosphorylating enzymes are distributed on the cell surface. He found that glucose, resulting from the degradation of cellulose, must be phosphorylated before passing into the cell. Rothstein and Demis (1953) presented evidence suggesting that the activation of the phosphohexokinase reaction by extra-cellular potassium is essential for the fermentation of glucose by the yeast cell.

Summary

A washed suspension of rumen microorganisms was utilized in studying the requirements of rumen microflora for sodium, potassium, rubidium, lithium and cesium in bringing about accelerated cellulose digestion. Interrelationships among these alkali metals were also studied. The presence of potassium in the fermentation medium was found to be essential for *in vitro* cellulose digestion whereas sodium was without effect in the absence of potassium. A study of interrelationships of varying concentrations of sodium and potassium showed that the addition of sodium to a fermentation medium containing 50 mcg. or less of potassium per ml. had either no influence or depressed cellulose digestion. However, when the potassium concentration was from 100 to 400 mcg. per ml. addions of sodium increased cellulose digestion. Chloride was not a limiting factor in these studies.

Rubidium was found to be capable of replacing approximately 50% of the potassium requirement for *in vitro* cellulose digestion, whereas lithium and cesium were ineffective.

The results of this work indicate that under the fermentation conditions employed a minimum concentration of 100 mcg. of potassium per ml. of fermentation medium is essential for *in vitro* cellulose digestion. Although sodium is not essential in the strictest sense, it appears desirable to maintain a certain ratio between the concentrations of sodium and potassium for maximum cellulose digestion. Responses in weight gain from sodium and potassium incorporated in a low-ash lamb fattening ration were in harmony with these results.

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UTILIZATION OF VITAMIN A IN DIFFERENT CARRIERS BY BEEF CATTLE

W. C. Sherman, G. O. Kuhn, E. A. White, W. D. Lewis, W. M. Reynolds and H. G. Luther

Chas. Pfizer & Co., Inc.

Terre Haute, Indiana

NATURE of the carrier is known to affect efficiency of utilization of vitamin A by several species. For humans, aqueous dispersions of vitamin A have been found superior to oil solutions for maintaining blood levels and promoting growth (Kramer, 1947; Lewis, 1947, 1950; Sobel, 1949; and their respective coworkers). Kagan *et al.*, (1953) reported that both alcohol and ester forms in aqueous dispersion enter the blood more readily than vitamin A in oil.

Aqueous forms of vitamin A have shown superior bio-availability to oil forms with dairy calves (Jacobson *et al.*, 1954; Wise *et al.*, 1949), with chicks (Halpern and associates, 1947) and with rats (Popper and Volk, 1948; Volk and Popper, 1950; Lewis and Cohlan, 1950; Sobel *et al.*, 1948). Vitamin A dispersed in gelatin beadlets has been found superior to oil solution for liver storage in rats and for growth and liver storage in chicks (Luther *et al.*, 1952), an observation which was recently confirmed in rats by Brüggemann and Tiews (1956) and in chicks by Ascarelli (1957).

Particle size of the vitamin A within the gelatin beadlet affects efficiency of utilization (Luther *et al.*, 1952). It was found that for rats particles of vitamin A of 2 microns or less in diameter dispersed in gelatin beadlets brought about 69% greater liver storage than was obtained with an oil solution. With 5-micron particle size the superiority was 24%, and with 20-micron particle size liver storage was approximately the same as with an oil solution.

The present investigation compares effectiveness for beef cattle of vitamin A in oil solution, in aqueous emulsion and dispersed in gelatin beadlets.

Experimental

Yearling Hereford steers were depleted of vitamin A by self-feeding ground corn cobs and 3 lb. per head per day of a low-vitamin A protein supplement of the following composition: Solvent soybean oil meal 63.8, dry molasses supplement (45% sugar) 28, steamed bone meal 6, trace mineralized salt (1.2 ounces of cobalt chloride added per 100 lb.) 1.7, and vitamin D supplement (1500 U.S.P. units per gm.) 0.5%. The supplement was fed in two equal portions in the morning and afternoon. The animals

were maintained on this feeding program until the blood plasma vitamin A was 10 mcg. per 100 ml. or less. They then were divided into groups for administration of vitamin A doses based on plasma level and body weight. The vitamin A preparations, in gelatin capsules, were administered by a balling gun, usually in the evening for convenience of blood sampling.

The vitamin A sources used were a pharmaceutical grade halibut liver oil (60,000 U.S.P. units per gm., Parke, Davis & Co.); a corn oil concentrate of synthetic vitamin A palmitate containing 1,680,000 U.S.P. units per gm.; an aqueous preparation of vitamin A palmitate prepared by dispersing the oil concentrate of pure synthetic vitamin A palmitate in water solution containing 15% Tween-80;* and Pfizer A-250 or A-250 P, containing 250,000 U.S.P. units of vitamin A per gm. as the pure acetate or palmitate dispersed in gelatin beadlets. In preparing the materials for administration the oil and aqueous preparations were diluted with corn oil and water, respectively. The capsules were filled with the aqueous preparation immediately before dosing. The vitamin A palmitate in gelatin was diluted with soybean meal to approximately 20 ml. volume to nearly fill the gelatin capsules. The preparations were analyzed for vitamin A by the Carr-Price colorimetric and U.S.P. XIV spectrophotometric methods just prior to administration. Dosage levels were based on the Morton-Stubbs corrected U.S.P. spectrophotometric values.

The schedule of dosing and plasma and liver sampling is given in the tables. Blood samples were taken from the jugular vein. Blood and liver samples from individual animals were analyzed for vitamin A using the Carr-Price reaction according to slight modifications of the methods of Kimble (1939) and Gallup and Hoefer (1946), respectively.

The individual blood plasma vitamin A responses at 12 hours and the average blood plasma responses for the total period were subjected to an analysis of variance. Normal assumptions were made that the error of assay was independent of time subsequent to treatment and that errors were randomly distributed.

Results and Discussion

Plasma vitamin A values obtained in eight experiments following several different dosages are presented in table 1. At the lowest dosage level, a single dose of 2800 I.U. per 100 lb. body weight (experiment 1), there was no appreciable rise in plasma vitamin A of animals given the oil solution, but there was an indication of a blood response at 12 hours from aqueous vitamin A and vitamin A in gelatin beadlets. The average response for the entire period was significantly greater for the aqueous and gelatin forms than for the oil solution. With a single dose of 5600 I.U. per 100 lb. body weight (experiment 2, table 1) there was a more rapid response to the

* Trademark Atlas Powder Co.

TABLE 1. CATTLE PLASMA VITAMIN A FOLLOWING ORAL ADMINISTRATION OF VARIOUS FORMS

Experiment no.	1			2		3		4		5		6		7		8	
Dose vitamin A, I.U./100 lb.	2,800			5,600		11,000	6,000	6,000		36,000 (5 da. doses)		72,000		36,000		36,000	
Form of vitamin A[a]	A	B	C	A	B	A	B	A	D	A	D	A	D	E	D	A	E
No. steers	2	2	1	2	2	2	2	3	3	3	3	3	3	3	3	6	6
Plasma vitamin A, mcg./100 ml.																	
Initial	9.45	4.55	7.60	7.60	8.60	6.55	7.90	3.50	2.87	5.03	4.03	11.70	13.03	6.43	6.17	4.60	4.95
12 hours	9.2	6.1	7.9	7.9	9.8	10.6	12.8	5.0	5.3	6.1	10.4	20.4	28.3	9.6	14.9	7.8	7.3
14 hours	10.4	5.8	11.3	9.4	10.0	9.7	13.0	4.9	5.5	...	...	...	...	8.8	12.2	...	...
16 hours	9.5	10.3	12.9	10.4	14.2	11.4	13.7	...	...	10.3	15.3	28.7	39.5	11.7	18.5	11.1	9.6
20 hours	12.8	8.8	12.8	12.2	14.6	14.6	14.2	...	...	13.7	19.7	38.5	45.7	13.4	25.3	15.5	15.8
24 hours	9.6	7.8	12.9	11.1	15.0	...	...	...	...	17.5	24.5	47.8	52.0	22.3	32.9	21.1	22.5
36 hours	...	...	...	12.7	17.8	17.1	19.0	5.7	6.8	30.1	35.7	47.6	45.3	36.6	37.1	32.9	36.6
48 hours	...	...	...	...	...	...	...	...	...	...	...	...	...	35.6	35.7	32.3	34.4
60 hours	...	...	...	11.7	16.8	15.2	21.8	9.0	9.5	...	...	...	...	32.1	32.5	28.5	30.7
84 hours	...	...	...	11.3	17.2[b]	14.6	18.1	8.1	12.1	29.4	36.0	28.7	27.2	26.2	27.6	23.1	27.1
132 hours	...	...	...	...	...	13.0	14.0	7.4[c]	7.3	...	...	...	...	...	...	...	...
170 hours	...	...	...	...	...	...	...	...	...	24.2	26.7	...	...	...	...	...	...
228 hours	...	...	...	...	...	10.2	11.6	...	...	...	...	...	...	...	...	...	...
336 hours	...	...	...	...	...	...	...	...	...	20.8	21.8	...	...	...	...	...	...
348 hours	...	...	...	...	...	8.7	9.9	...	...	...	...	...	...	...	...	...	...
1056 hours	...	...	...	...	...	...	...	...	...	20.8	21.1	...	...	...	...	...	...
Av. plasma response mcg./100 ml.																	
12 hours	−0.3	1.55	0.3	0.3	1.15	4.05	4.90	1.50	2.43	1.07	6.37	9.14	15.3	3.17	8.73*	3.10	2.35
Total period[d]	0.85	3.22*	3.96*	3.24	5.83*	5.97	6.90	3.18	4.88*	14.18	19.44*	23.63	26.67	15.71	20.21*	16.59	18.05
Index (12 hours)	...	...	...	100	384	100	222	100	162	100	596	100	168	100	275	100	82.5
Index (total period)	100	375	461	100	180	100	212	100	154	100	137	100	113	100	129	100	109
L.S.D. (12 hours)*	4.53			4.16		8.80		2.66		6.76		12.15		4.99		4.66	
L.S.D. (total period)*	2.02			1.56		2.78		1.09		2.26		4.96		1.66		1.65	

[a] The forms of vitamin A used were:
A=Vitamin A palmitate in oil
B=Vitamin A palmitate in aqueous dispersion
C=Vitamin A actetate in gelatin beadlet
D=Vitamin A palmitate in gelatin beadlet, Pfizer A–250P
E=Haliver oil, pharmaceutical grade

[b] 84-hour value in experiment 2B represents one animal.

[c] 132-hour value in experiment 4A is an average of two animals.

[d] Values for the average response, total period, are the difference between the initial plasma vitamin A and the average of all values after the initial.

* Significantly different from controls, P=0.05.

aqueous dispersion than to the oil solution and a significantly greater average response in the animals which received the aqueous dispersion.

When aqueous dispersion (6000 I.U. per 100 lb.) and oil solution (11,000 I.U. per 100 lb.) were compared (experiment 3) the 12-hour plasma response and the average total period response were equal to those produced by the oil even though the level of vitamin A administered in oil was nearly double the level in aqueous dispersion.

A comparison of vitamin A palmitate in oil and in gelatin beadlets, both at 6000 I.U. per 100 lb. body weight (experiment 4), showed a greater increase in plasma A at 12 hours in the animals given the gelatin source as well as a significantly higher average response in plasma vitamin A.

TABLE 2. LIVER VITAMIN A OF STEERS FOLLOWING ADMINISTRATION OF VITAMIN A IN OIL AND GELATIN BEADLETS

Source of vitamin A [a]	No. steers	Mcg./gm. Liver *		
		Before dosing	Days after initial dose	
			7	24
Palmitate in gel. beadlets	2 [b]	0.125	12.4	5.0
Palmitate in oil	3	0.24	9.2	4.7

* L.S.D.=6.27 for the combined 7- and 24-day values.

[a] Vitamin A was given in five consecutive daily doses of 36,000 I.U./ 100 lb.

[b] There was difficulty in obtaining the 7-day liver sample in one of the three animals which received vitamin A palmitate in gelatin. Liver values for this animal were therefore not included in the average.

With doses of 36,000 I.U. per 100 lb. body weight daily for 5 successive days (experiment 5) the gelatin beadlet form brought a pronounced increase in plasma content in 12 hours after administration of the first dose, whereas oil solution brought no appreciable increase by this time. The plasma vitamin A of animals given the gelatin beadlets was maintained at a higher level than that of those given the oil solution for 84 hours, after which the differences tended to diminish.

With a single dose of 72,000 I.U. per 100 lb. (experiment 6) there was a greater response at 12 hours and for the entire period in the animals given the vitamin A in gelatin than in those given the oil, although the differences were not statistically significant. The higher level of plasma vitamin A in the steers given the vitamin in gelatin beadlets persisted for approximately 24 hours, after which the difference disappeared.

A direct comparison of synthetic vitamin A palmitate in gelatin and natural vitamin A in haliver oil was made in experiment 7, both forms being given in a single dose of 36,000 I.U. per 100 lb. The responses with the synthetic vitamin A in gelation at 12 hours and for the entire period were significantly greater than the responses with fish liver oil.

In experiment 8 oil solutions of natural vitamin A and synthetic vitamin A were compared to determine whether differences in the isomeric forms of vitamin A occurring in natural and synthetic vitamin A could partially account for the differences in the blood responses obtained in experiment 7. Rat assays in support of the latter possibility show that neo-vitamin A (2-mono-*cis*), which is present in appreciable amounts in natural sources, is only 75% as potent for rat growth and liver storage as the all-*trans* form of vitamin A (Ames *et al.*, 1955) occurring in synthetic vitamin A. The results of this comparison with cattle (experiment 8) show no significant differences in the 12-hour and total period responses for the oil solutions of the synthetic and natural vitamin A.

It is possible, however, that small differences in bio-availability of the natural and all-*trans* synthetic vitamin A could have been masked by the non-vitamin A impurities in the fish liver oil in experiments 7 and 8. The analytical values obtained on the fish liver oil by the Carr-Price and U.S.P. spectrophotometric methods were in a ratio of 1.26, whereas the corresponding Carr-Price: U.S.P. spectrophotometric ratios for the pure synthetic vitamin A in gelatin and in corn oil were all within a range of 1.00–1.05. Assuming that the Carr-Price reacting impurities of the fish liver oil were absorbed by the animals, with dosage levels based on corrected U.S.P. spectrophotometric analyses, the blood plasma vitamin A values determined by the Carr-Price method could have included impurities from fish oil which would not have been present in the plasma of animals treated with synthetic vitamin A.

In consideration of the effect of level of administration of vitamin A upon the sensitivity of the animals to differences in bio-availability of different forms of vitamin A, the data clearly indicate that for the total-period-plasma response, the differences are greater at the lower levels of vitamin A administration and nearly disappeared with the highest level of 72,000 I.U. per 100 lb. For differences in the initial appearance of vitamin A in the plasma at 12 hours, difference in dosage level did not appear consistently to influence the results although at the lowest level, 2800 I.U. per 100 lb., the response curve was too gradual for differences to be demonstrated.

Liver storage studies were carried out in the cattle used in experiment 5. Liver samples of approximately 2 gm. size were removed by a biopsy instrument just before dosing, at 7 days (2 days after the animals had received the last of five daily doses of 36,000 I.U. vitamin A per 100 lb.) and 24 days after the initial dose. The initial liver content of vitamin A averaged less than 0.25 mcg. per gm. in both groups of cattle. The average 7-day liver vitamin A of the animals given vitamin A in gelatin was over 30% higher than the corresponding value for the group which received an oil solution. Due to variability in the animals in liver responses and the

limited number of animals, however, this difference was not statistically significant although the difference in total blood response was significant.

Summary

1. Vitamin A depleted cattle were given orally pure synthetic vitamin A esters in the forms of aqueous dispersion, oil solution and gelatin beadlets at levels of 2800–72,000 I.U. per 100 lb. body weight. The aqueous and gelatin beadlet forms of vitamin A produced greater blood plasma vitamin A responses at 12 hours after dosing and greater average plasma responses for the entire period following dosing than did the oil solutions of vitamin A.

2. Oil solutions of synthetic vitamin A and natural vitamin A (fish oil) when administered in equal doses produced blood plasma responses which were not significantly different at 12 hours or for the entire period.

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A COMPARISON OF FIVE METHODS OF ESTIMATING CLEAN FLEECE WEIGHT

GEORGE M. SIDWELL,[1] P. E. NEALE [2] AND GORDON L. JESSUP, JR.[1]

United States Department of Agriculture and New Mexico Agricultural Experiment Station

THE selection of individual sheep on the basis of clean wool production is of primary importance in a wool improvement program. This selection has been neglected because of the difficulties involved in measuring this important trait. In most cases, scouring the whole fleece is too costly and impracticable.

Schott and coworkers (1942), Pohle and Hazel (1944) and Pohle, Hazel and Keller (1945) found small sample scouring to be very accurate in estimating whole fleece clean weight. However, like whole fleece scouring, small sample determinations are costly and time consuming. Terrill *et al.* (1945), Ali *et al.* (1953), Fox and Esplin (1954), and Sidwell and coworkers (1956) have shown that clean fleece weight can be predicted from other traits, mainly staple length, grease fleece weight, body weight and wool density. However, these prediction equations will probably have reduced accuracy when applied to flocks other than the one from which the data were obtained and will be still less useful for comparisons among sheep kept in different flocks.

In view of these difficulties involved in the measurement of clean fleece weight, Professor P. E. Neale of the New Mexico College of Agriculture and Mechanic Arts has developed a machine with which to estimate clean fleece weight. This method of determination has been shown to be accurate (Neale, Sidwell and McFadden, 1956), very rapid and to require no technical knowledge or skill. It is the purpose of this work to compare this method with other methods of determining clean fleece weight.

Material and Methods

The five methods of estimating clean fleece weight which are compared in this study are: (1) Whole fleece scouring, (2) small sample scouring, (3) machine method, (4) multiple regression equations involving staple length, grease fleece weight, body weight and wool density, and (5) a method using these same four traits described by Neale and McFadden (1954).

The data used in this study were collected from 139 yearling ewes and 61 yearling rams representing all of the yearling sheep raised in 1956 at

[1] Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico, in cooperation with the New Mexico College of Agriculture and Mechanic Arts.

[2] Animal Husbandman, New Mexico College of Agriculture and Mechanic Arts, State College, New Mexico.

the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico. These sheep represent eight different breeding groups but for the purpose of this work they can be divided into two classes on the basis of wool grade; namely, those with wool grading ⅜ blood and coarser and those with wool grading ½ blood and finer.

Just prior to shearing in April, a small sample of wool weighing about 40 gm. was taken from the left side of each sheep. This sample was immediately placed in a moisture proof container, being scoured later in a soap and soda ash solution and dried to an oven-dry basis, then adjusted to standard conditions. At the time the wool samples were taken a small

TABLE 1. MEANS OF STAPLE LENGTH, BODY WEIGHT, GREASE FLEECE WEIGHT AND WOOL DENSITY, BY WOOL TYPE AND SEX

Items	No.	Staple length (in.)	Grease fleece weight (lb.)	Body weight (lb.)	Wool density (units)
Fine wool					
Rams	33	2.86	6.69	104	346
Ewes	102	2.74	5.46	67	330
Coarse wool					
Rams	28	4.53	6.14	107	315
Ewes	37	3.87	4.33	65	300

lock of wool was also clipped off the left midside close to the skin for staple length determination. Each sheep was individually weighed to the nearest whole pound. The wool density was determined with the use of the Neale Density Meter described by Ali, Neale and McFadden (1953). At shearing time each fleece was weighed to the nearest 0.05 lb. then wrapped in cheesecloth to maintain its identity. These fleeces were scoured at the New Mexico College of Agriculture and Mechanic Arts wool laboratory. After scouring each fleece was corrected for residual grease, vegetable matter and ash, and adjusted to standard conditions.

Results and Discussion

The means of clean fleece weight by sex and wool type as estimated by each of the five methods are shown in table 2. Using the clean fleece weight determined by scouring the whole fleece as an actual or true weight, with which to compare the other estimates, it can be seen that all estimates except that obtained from scouring small samples predict this weight very closely. The small sample value has tended toward a slight over-estimate in each case except for the fine wool ewes. Schott and coworkers (1942) found this same thing to be true in the coarse wool fleeces with which they worked. However, they also found that

TABLE 2. MEANS OF CLEAN FLEECE WEIGHT AS ESTIMATED BY FIVE METHODS

Items	Whole fleece scouring	Small sample scouring	Machine	Multiple regression	Neale method
Fine wool					
Rams	3.35	3.69	3.36	3.34	3.41
Ewes	2.61	2.60	2.59	2.61	2.71
Coarse wool					
Rams	3.73	4.17	3.85	3.73	3.79
Ewes	2.60	2.93	2.60	2.62	2.71
Overall average	2.89	3.06	2.90	2.89	2.98

this over-estimate could be corrected by dividing the percent yield minus the "a" value associated with the regression for a particular type of wool by the overall regression coefficient of whole fleece clean yield on sample clean yield. This same correction when applied to these data brought the sample estimate means of the coarse wool closer to the mean of the whole fleece clean weight.

It should be remembered that all of the methods of obtaining clean fleece weight used in this study except the small sample estimate involve the use of the clean fleece weight obtained from scouring the whole fleece, and are thus based on the actual data to which they are applied. For this reason the other three methods should estimate the average whole fleece value very closely.

Table 3 shows the multiple regression equations used to predict clean fleece weight for each sex and wool type. Also included in table 3 are the

TABLE 3. PREDICTION EQUATIONS

Animals	Regression of clean fleece weight on: Body weight	Wool density	Staple length	Grease fleece weight	"a" values	R[a]
Fine wool ewes	0.0143	0.0018	0.5189	0.2456	—1.71	0.82
			.4997	.3353	—.59	.81
Coarse wool ewes	.0144	—.0005	.2206	.4490	—.95	.93
			.2062	.5272	—.45	.92
Fine wool rams	.0046	.0059	.4347	.3576	—2.81	.96
			.2922	.4526	—.51	.93
Coarse wool rams	—.0005	—.0001	3213	.5328	—.90	.95
			.3219	.5289	—.96	.93

[a] Mutiple correlation coefficients.

multiple correlation coefficients (R) and the "a" values for each equation.

Since body weight and density appeared to add very little accuracy to the equations, new regression coefficients for staple length and grease fleece weight were obtained, leaving out body weight and density. As can be seen by the multiple correlation coefficients little information is lost if these two traits, body weight and wool density, are omitted.

Table 4 shows the correlation coefficients between the clean fleece

TABLE 4. CORRELATION COEFFICIENTS

Items	Grease fleece weight	Small sample clean weight	Machine method clean weight	Multiple regression clean weight	Neale method clean weight
Whole fleece clean weight	0.80	0.92	0.87	0.95	0.87
Grease fleece weight		.75	.78	.82	.79
Small sample clean weight			.85	.95	.84
Machine method clean weight				.91	.80
Multiple regression clean weight					.95

weights obtained by each of the five methods of estimating clean weight and between each method and grease fleece weight. In these data the most accurate estimate is that obtained from the use of the regression equations as evidenced by the high correlation of 0.95 between the whole fleece scouring clean weight and the estimate obtained from the use of the regression equations. However, the small increase in accuracy over the machine method does not compensate for the many advantages which the machine method possesses. First, there is a great saving of time as the machine method requires less than 2 min. per fleece. Second, the estimate is independent of any other trait or measurement. Third, no distinction need be made between ram or ewe fleeces. Fourth, no technical skill or knowledge is required for its operation. Fifth, this method is applicable to any flock. It is doubtful that the regression equations obtained for these sheep in this study can be applied to sheep in other flocks unless those other sheep fit very closely the description of the sheep in this study.

Although the correlation between grease fleece weight and clean fleece weight is reasonably high, it is still low enough that selection for clean wool production should be made on the basis of the amount of clean wool produced by the individual sheep rather than on grease fleece weight for maximum improvement in clean wool production.

Summary

Clean fleece weight obtained by scouring the whole fleece from a total of 139 yearling ewes and 61 yearling rams was compared to (1) the results obtained from scouring small side samples, (2) multiple regression equations predicting clean fleece weight from wool density, body weight, staple length and grease fleece weight, (3) a method described by Neale and McFadden (1954) and (4) the results obtained from a new machine designed by P. E. Neale for estimating clean fleece weight. All methods of estimation appear to be reasonably accurate as shown by the correlation coefficients between whole fleece scouring and each of the four methods described above. These correlations were 0.92, 0.95, 0.87, and 0.87, respectively. It was shown by the multiple correlation coefficients that very little information concerning clean fleece weight is lost in predicting clean fleece weight by the multiple regression method if wool density and body weight were omitted from these equations. The small increase in accuracy of the small sample and multiple regression methods over the machine method does not compensate for the saving of time and expense involved as the machine method requires less than 2 min. per fleece for the estimate. The machine requires no technical skill or knowledge for its operation and is applicable to all flocks. Also, the clean fleece weight estimate derived from the machine is independent of all other traits.

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FERTILIZATION RATES OBTAINED WITH BOAR SEMEN STORED FOR ZERO AND TWELVE HOURS[1, 2]

FREDERICK W. STRATMAN, H. L. SELF AND VEARL R. SMITH[3]

University of Wisconsin,[4] Madison

SEVERAL workers have reported that unsatisfactory results were obtained when swine were inseminated artificially with diluted or stored boar semen (Lasley, J. F., 1943; Ito *et al.*, 1948; Polge, C., 1956; Mesnil Du Buisson *et al.*, 1956). In general, the above workers and others (Rodin *et al.*, 1936; Wiggins *et al.*, 1951) obtained satisfactory results when comparatively large volumes of fresh semen was used.

More satisfactory methods for the handling and storage of boar semen will have to be developed before artificial insemination in swine can become practical. Preliminary studies were made to determine which diluent or combination of diluents was best for maintaining and prolonging sperm motility of boar semen. A modified Kreb's solution (Lardy and Phillips, 1942), egg yolk citrate of the same composition as used for dilution of bull semen (Salisbury *et al.*, 1941; Swanson, E. W., 1949) and homogenized milk as prepared for dilution of bull semen (Thacker and Almquist, 1951) were compared. These comparisons indicated that type and percent motility were best maintained in semen fractionated by centrifugation and diluted with milk. Subsequently an experiment was designed to ascertain the suitability of whole homogenized milk as a diluent for boar semen.

Methods and Materials

Four Chester White boars penned together and approximately 8 months of age provided semen during October and November, 1956. In no case were collections made from the same boar more often than once in 3 days. Semen was collected by means of an artificial vagina. An estrual gilt was used as a teaser.

Semen was examined soon after collection at a magnification of 430X to determine the percentage of motile sperm and the type of motility. The percentage of motile sperm was rated on a scale of 0 to 100 and on type of motility on a scale of 0 to 10. A rating of 10 denoted a vigorous straight forward movement with gradations down the scale to 1 which denoted a weak, slow and spasmodic movement of the sperm.

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[3] Presently, Head, Department of Dairy Science, University of Arizona.
[4] Departments of Animal Husbandry and Dairy Husbandry.

Semen volume was measured immediately following collection and also after straining through four layers of 44 x 36 mesh gauze to remove the gelatinous portion. The ejaculate was then divided into two portions. The "fresh" semen portion of the strained ejaculate was prepared by diluting 20 ml. of the fresh semen to a total volume of 50 ml. with a milk diluent. The fresh semen was used for inseminating a gilt within 5 to 10 minutes after the collection was completed. The "stored" semen portion of the strained ejaculate was prepared by centrifuging 20 ml. for 7 minutes at 1500 r.p.m. in a centrifuge with a diameter of 27.5 cm. After centrifugation, the supernatant fluids were poured off and the remaining semen rediluted to the original volume (before centrifugation) of 20 ml. with a milk diluent. The "stored" semen was then held at 9° C. for approximately 12 hours. After storage, but before final dilution, the "stored" semen was again evaluated for percentage and type of motility. The semen was warmed to body temperature and examined as described above. Immediately prior to insemination, the "stored" semen was diluted to a total volume of 50 ml. with a milk diluent.

The diluent used was whole homogenized milk that had been heated to a temperature of 93° C. for approximately 10 minutes. Dihydrostreptomycin sulfate was added to the diluent at the rate of 1,000 mcg. per ml. Whenever a dilution was made, the diluent and semen were brought to the same temperature.

Forty gilts of mixed breeding, weighing 200 to 220 lb. whose sexual age (number of previous heat periods) was unknown, were paired according to the sequence in which they exhibited estrus. The first gilt of a pair preceded the second gilt of the pair by an average of approximately 12 hours in the onset of estrus. Semen was collected when the first member of a pair was in estrus and that gilt was inseminated with "fresh" semen. The second member of the pair was inseminated with the "stored" semen from the same ejaculate. The same volume of diluted semen, 50 ml., was used for inseminating each member of a pair.

The gilts were checked for estrus with a vasectomized boar at 12-hour intervals. A gilt was considered in estrus when she allowed the boar to mount. Service by the boar was not permitted so as to avoid possible stimulation and uterine contamination. Gilts were artificially inseminated approximately 12 hours after the first standing heat, i.e., 12 to 23 hours after the onset of estrus. A plastic tube 18 in. long and 5 mm. in diameter as used for inseminating cows was modified to simulate the anatomy of the boar penis to facilitate entry into the cervix, The tube was attached to a 50 ml. glass syringe by means of a short length of rubber tubing. The semen was deposited in the cervix in all inseminations.

Each gilt was slaughtered 3 to 4 days after insemination—12 to 48 hours after the end of estrus. The oviducts and uterine horns were flushed with a 0.9% saline solution. The washings were collected in a watch glass and examined under a steroscopic microscope at a magnification of

90X to determine the number of ova recovered and percent fertilized. An ovum was considered to be fertilized if it had two or more blastomeres similar in size. Percent fertility was the percentage of the eggs recovered that were cleaved. Conception rate was the percentage of gilts bred that had some cleaved ova.

The percentage of ova recovered was the ratio of the number of ova recovered to the number of corpora lutea.

The data were examined statistically as outlined by Snedecor (1953).

Results and Discussion

The percent fertility for "fresh" and "stored" semen using split ejaculates is shown for different boars in table 1. An analysis of variance indicates there were no significant differences among the boars or between the fresh and stored semen. Neither were there differences among ejaculates within boars.

The mean percent fertility for all fresh semen samples was 76.9% as compared to 83.6% for the stored samples. The overall mean of 80.2% is below the value of 91.0% reported by Wiggins *et al.* (1951) for 10 gilts inseminated with 20 ml. of fresh semen diluted to a total volume of 50 ml. with a modified Kreb's solution (Lardy and Phillips, 1942). It is, however, comparable to the value of 82.0% reported by Warnick *et al.* (1951) with gilts bred by natural service. It is lower than the values reported by several other workers (Hauser *et al.*, 1952; Robertson *et al.*, 1951; Self *et al.*, 1955; Squiers *et al.*, 1952) for gilts bred by natural service.

In the group inseminated with "fresh" semen 18 of 20 gilts (90%) had one or more fertilized ova and 19 of 20 gilts (95%) inseminated with "stored" semen conceived (table 1). The mean conception rate of 92.5% for the 40 gilts is lower than the 100% conception rate reported by Wiggins *et al.* (1951), but it is higher than the 74% reported by Squiers *et al.* (1952) for gilts bred by natural service and slaughtered approximately 3 days later.

The conception rate as established by the presence of fertilized ova at 3 to 4 days after breeding will be higher than a conception rate established at a later stage of gestation. This is due to a somewhat continuous occurrence of embryonic death during gestation (Corner, 1923). Casida (1953) estimated 36.7% of the pig embryos actually formed died before birth. Thus a direct comparison of data obtained at the third or fourth day of gestation with data obtained at other stages is not feasible but it should be noted that Lasley (1943) reported only 7 of 30 gilts (23%) farrowed when inseminated once during estrus with semen stored 24 hours; Ito *et al.* (1948) reported 76.8% of the gilts farrowed when inseminated with semen stored at a temperature of 15–20° C. for 0 to 24 hours; Polge (1956) reported a 50% farrowing rate when gilts were inseminated with 100 ml. of diluted semen that had been stored 2 to 6

TABLE 1. A COMPARISON OF FRESH AND STORED SEMEN USING SPLIT EJACULATES

Boar no.	Treatment group	Percent fertile ova [a]					Percent conception [b]	Semen ratings			
		0	1–49	50–99	100	Average		Motile, % Mean	Motile, % Range	Motility type Mean	Motility type Range
1	Fresh (5) [c]	1 [d]	..	1	3	77.8	80.0	70	65–80	7.8	6–9
	Stored (5)	..	..	3	2	88.0	100.0	47	35–55	5.4	4–7
2	Fresh (5)	1	1	..	3	61.8	80.0	62	50–70	7.0	6–8
	Stored (5)	..	..	2	3	87.3	100.0	46	35–55	4.8	4–5
3	Fresh (5)	..	1	1	3	81.2	100.0	78	65–85	7.4	7–8
	Stored (5)	1	..	1	3	78.6	80.0	44	30–60	5.4	5–7
4	Fresh (5)	..	..	2	3	87.0	100.0	79	75–85	8.2	7–9
	Stored (5)	..	1	2	2	80.7	100.0	52	45–65	5.2	4–6
All Boars	Fresh (20)	2	2	4	12	76.9	90.0	72.2	50–85	7.6	6–9
	Stored (20)	1	1	8	10	83.6	95.0	47.2	30–65	5.2	4–7
	Overall (40)	3	3	12	22	80.2	92.5	59.8		6.4	

[a] Percent fertile ova $= \frac{\text{No. fertile ova}}{\text{No. ova recovered}} \times 100$

[b] Percent conception $= \frac{\text{No. gilts with one or more cleaved ova}}{\text{Number of gilts inseminated}} \times 100$

[c] No. in parenthesis denotes number of gilts inseminated per boar-treatment group.

[d] Indicates number of gilts in each percent-fertile-ova subgroup.

hours and only 33.3% when 100 ml. of diluted semen, stored 24 to 30 hours, was used.

The mean percentage of ova recovered for the 40 gilts was 78.1%. This is approximately the same as the 78.9% reported by Hauser *et al.* (1952) and the 80.3% by Squiers *et al.* (1952) but it is lower than 86.0% recovery rate for 35 gilts obtained by Baker *et al.* (1954).

The percent motile sperm and the type of motility is shown in table 1. The mean percentage motile sperm for fresh semen and stored semen was 72.2% and 47.2%, respectively, while type of motility was 7.6 and 5.2. There was considerable reduction in motility during the 12 hours of storage, yet the fertilizing capacity remained approximately the same for the semen stored 12 hours as for the control samples of fresh semen.

The association in the fresh semen between the percent motile sperm and the percent of fertilized ova was not significant ($r=0.14$). but the association of type of motility in the fresh semen samples and the percent of fertilized ova was highly significant ($r=0.89$, $P<0.01$). Thus, type of motility of fresh semen appears to be a better indicator of the fertilizing capacity of spermatozoa than is the percentage of sperm that exhibit motility. The regression of fertility on type rating indicated that for each unit decrease in the type rating there was an accompanying decrease of 7.35 percentage points in percent fertility of fresh semen samples.

Neither percent motile sperm nor type motility in the stored semen samples was significantly associated with the percent fertile ova ($r= -0.30$ and -0.08, respectively). Under the conditions of this experiment, the percent motile sperm and the type of motility do not appear to be a good criterion for estimation of the fertilizing capacity of sperm stored for 12 hours at 9° C.

The significant association between type of motility and percentage fertility for fresh semen and the lack of association of these factors for stored semen indicates that the same method of evaluation is not equally suitable for these two kinds of boar semen samples. It is not readily apparent why whole milk is capable of maintaining the fertilizing capacity of boar sperm for at least 12 hours without also maintaining satisfactory motility values.

Summary

Twenty pairs of gilts were artificially inseminated from split ejaculates with fresh semen or semen stored for 12 hours. Observations were recorded showing the type and percent motility, the percent of gilts conceiving, and the percent of recovered ova that were fertilized. An analysis of variance indicated there were no significant differences among boars, among ejaculates within boars or between fresh semen or semen stored for 12 hours. The average percent fertility for all fresh semen samples was 76.9% as compared to 83.6% for the stored samples, while the

average conception rate was 90.0% and 95.0%, respectively. The association between the percent motile sperm in the fresh semen and the percent fertile ova was not significant, but the type of motility and percent fertile ova were significantly related ($r=0.89$, $P<0.01$). These factors were not significantly associated in the stored semen.

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THE ACCURACY OF WEIGHTS AND GAINS OF BEEF CATTLE

ROBERT M. KOCH, E. W. SCHLEICHER AND VINCENT H. ARTHAUD

University of Nebraska and
United States Department of Agriculture [1,2]

THE accuracy of cattle weights is of interest to many segments of the livestock industry. Recommendations based on experimental studies in breeding, feeding and management are based on comparisons of live animal weights or gains. It is well recognized that cattle weights fluctuate from day to day. The recommended procedure of weighing cattle for three consecutive days (Lush *et al.*, 1928) to remove inaccurate appraisal of live animal weights has been followed by most experimental workers. More recent studies by Bean (1946, 1948) on swine and sheep and by Patterson (1947) and Baker *et al.* (1947) on cattle indicate that under certain conditions the practice of weighing for three consecutive days does not justify the expense and trouble of taking the extra weights.

The increasing acceptance of performance tests for differences in gaining ability has focused considerable interest on weights and gains of individual animals. The error associated with the live animal weight or gain of individuals is important in judging whether observed differences are likely to be real or due to chance variation.

This paper seeks to evaluate the relative accuracy of weighing cattle under full and shrunk conditions. It further seeks to evaluate the relative importance that differences in animals, days and chance fluctuations make in the observed variation and to compare the accuracy of single and multiple weights of cattle.

Data. The data for this study were obtained from the experimental breeding herds at the Nebraska Agricultural Experiment Station, Lincoln, and the Beef Cattle Research Station, Fort Robinson, Nebraska, during the years 1951, 1952, 1953 and 1954.

The weights are the three initial and three final weights of a 168-day feeding test. The three weights of a given series were taken one week apart and not on consecutive days and read to the nearest 2-pound interval. The initial weights were of bull and heifer calves that had been weaned the previous month and were given a 21-day adjustment period before starting with the three-weight series. At Lincoln, calves were weaned in two groups each year. At Fort Robinson, all calves were weaned in one group each year. The feeding system used was individual self-

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feeding. At Lincoln, a growing-fattening ration was fed, while at Fort Robinson the cattle received chopped hay with no concentrates. In the 1951–52 test, calves were tied to their individual feeders for a 2-hour period in the morning and a similar period in the afternoon. Weights for the full basis were obtained by weighing the calves directly out of the feedlots shortly after noon of the weigh day. Following this weighing the calves were allowed to eat and drink. At the close of the day the water tanks were covered or drained. The weights for the shrunk basis were obtained the following morning before feeding and after a 12- or 14-hour shrink. The management was changed in the 1952–53 and 1953–54 tests. The calves were tied to the individual feeders in the evening and turned out in the morning, necessitating a change in the weighing schedule. The full weights were obtained in the afternoon taking the cattle directly from the feedlots where they had access to water. The weights for the shrunk basis were obtained the following day. The calves were allowed to drink in the morning for about one hour after being turned out. The tanks were then covered or drained. The cattle were weighed in the afternoon after a 6- or 7-hour shrink. Data were not available for comparing full and shrunk weights on the Fort Robinson heifers for 1953–54.

Sources of Variation. The observed weight of an animal can, for practical purposes, be considered as the sum of body tissues plus the content of the digestive tract or fill. Ideally, comparisons of animal weights and gains would be based on differences in body tissue. However, this ideal situation cannot be realized and the comparisons in this report are based on body tissue plus a certain amount of fill. A reduction of differences in fill would allow a more accurate appraisal of differences in body tissue.

When three weights are obtained on cattle under similar treatment, the observed variation can be partitioned into differences between animals, days and residual amount. Animal differences are the differences in weight of body tissue and an average amount of fill. Each of these components is influenced by genetic differences affecting growth, differences in ages of the animals when weighed, and differences in environmental conditions affecting each animal.

The variation associated with days is the average change in weights of all animals from one day to the next. These changes may be caused by growth, changes in temperature, feed, excitement, and unknown influences peculiar to the day. Where weights are taken over a 3-day interval, the daily fluctuations would not be expected to contain much variation due to changes in body tissue but primarily changes in fill. Where weights are taken over a 2-week period, as in these data, part of the variation associated with days would be due to gains or losses in body tissue and part to fluctuations in fill common to all animals weighed on these days. The average gain or loss in body tissue and fill for all animals over the weighing period can be removed by partitioning the variation associated with days into that attributable to regression of weight of

all animals on days and the deviations of daily means from regression. The portion attributed to regression would contain the variation due to average growth over the weighing interval and changes in fill due to trends in evironmental conditions. The deviations from regression approximate the variation due to changes in daily conditions. The deviations underestimate daily fluctuations to the extent that the trends in weight are due to trends in environmental conditions and overestimate them if growth is curvilinear over the two-week period.

Residual variation measures the differential response of an animal from one weigh-day to the next that cannot be accounted for by the average change of all animals. The differential response of animals includes differences in growth rate of the individuals as compared with the average growth of all animals, fluctuations in fill not accounted for by differences in days, and inaccuracies of the scale or scale operator.

Analysis of Data. The data were analyzed separately for each sex, year and station combination. The variation associated with differences in sex and year were avoided by this grouping and left unanalyzed since they were not pertinent to this study. At Lincoln, the within year and sex groups were further divided into groups weaned on the same day since calves were weaned in two groups each year. The sources of variation studied were differences between animals, days and the residual portion.

The within subclass sums of squares were pooled for the various years but not for station or sex. The components of variation for animals, days, and residual were estimated by equating the expected value of the mean squares to the observed values. The estimates for these components are presented in table 1. The variation associated with the two degrees of freedom for differences in days was partitioned into a portion attributable to the trend in weights and the deviations from the trend. Only the portion associated with deviations from the linear trend are shown in table 1 because this estimates the fluctuations in weight due to days. The degrees of freedom for days represents the pooling of 6, 6, 3 and 2 subgroups of data each with one degree of freedom.

Discussion

The residual or error component of variance found in this study is larger than that reported by Lush *et al.* (1928) and Patterson (1947). One reason for the larger estimate of error may be the difference in the time interval between weights. In their studies the three weights were obtained on consecutive days whereas in these data the weights were taken a week apart. A study of daily variations in cattle weights by Baker and Guilbert (1942) indicated that daily deviations from the linear trend in weight were not at random but tended to be correlated, giving a cyclical appearance to the growth curve. An animal that had an above average fill on the first weigh day would also tend to have an above average fill

TABLE 1. COMPONENTS OF VARIANCE FOR INITIAL AND FINAL WEIGHTS ON A FULL AND SHRUNK BASIS (POUNDS)

Source of variation	d.f.	Initial weights		Final weights	
		Full	Shrunk	Full	Shrunk
Lincoln					
Bulls					
Days	6	68	31	32	37
Animals	120	2548	2519	7003	6805
Residual	240	85	83	137	92
Average weight, lb.		393	387	765	748
Heifers					
Days	6	56	11	9	13
Animals	90	2310	2292	4188	4181
Residual	180	61	58	66	60
Average weight, lb.		358	351	655	644
Fort Robinson					
Bulls					
Days	3	203	42	79	135
Animals	218	4158	3631	6778	6249
Residual	436[a]	106	77	207	160
Average weight, lb.		404	396	597	579
Heifers					
Days	2	11	17	90	10
Animals	137	4435	4244	5813	5481
Residual	274[b]	60	61	154	95
Average weight, lb.		375	366	512	497

[a] Full weight based on 395 d.f.
[b] Full weight based on 217 d.f.

on the following day. Where an interval of 7 days separates the weigh days there is more opportunity for an animal to change the amount of fill it contained on the previous weigh day. The error variation calculated from three weights on consecutive days would tend to underestimate the fluctuations in fill on the same animal. Whether one week is a sufficient time interval is not known. It is assumed that weights taken at weekly intervals are more independent and consequently are a better measure of the differences in fill than weights taken on consecutive days.

The measure of accuracy used in this study for comparing various systems of weighing was based on the relative size of the variances involved, since Fisher (1938) has shown that the precision of maximum likelihood statistics is given by the reciprocal of the variance.

Full Weights vs. Shrunk Weights. The residual components of variation shown in table 1 estimate the experimental error involved when testing differences between animals raised under similar conditions and weighed on the same day such as the weights of animals in a record of performance program. The residual components of variance on a shrunk

basis were smaller than those on a full basis for initial weights in three of the four groups presented in table 1. Only one of these comparisons was statistically significant, as determined by an *F* test at the 1% level of probability. The initial weights on a shrunk basis were 2, 5 and 27% less variable than the full basis for the first three groups of data in table 1. The full weights for the Fort Robinson heifers were 2% less variable than the shrunk weights. In the final weight series the residual variance was smaller for the shrunk basis in all four groups of data, three of these being statistically significant at the 1% level of probability. Expressed on a percentage basis the shrunk weights were 33, 9, 23 and 38% less variable than the full weights for the four groups of data.

The experimental error for comparisons where treatments (such as feeding trials) rather than animals are the primary criteria of classification involve the variance component between animals as well as the residual component. The experimental error in such cases is the sum of the animal and residual components. The experimental errors for the full basis in the initial weight series were 2633, 2371, 4264 and 4495 for the four groups shown in table 1. The comparable figures for the shrunk basis were 2602, 2350, 3708 and 4305, respectively. The reduction in experimental error due to shrinking was 1, 1, 13 and 4%. Similarly the experimental errors in the final weight series were 7140, 4254, 6985 and 5967 on a full basis and 6897, 4241, 6409 and 5576 on a shrunk basis. The reduction in experimental error due to shrinking was 3, 0, 8 and 6% for the four groups, respectively. None of the differences was statistically significant. Animal differences were less under the shrunk basis but shrinking did not reduce these differences as much as it did fluctuations in fill. The weight of body tissue was likely the same for both shrunk and full weights. The decreased variation between animals is assumed to be due to a smaller amount of fill. Whiteman *et al.* (1954) studying gains of grazing steers stated, "There was an indication that under some conditions the differences in amount of fill among animals tend to be lessened by shrinking".

The estimates of variation associated with days are not consistent. The particular set of days on which the estimates are based seem far more important than the influence of shrink, although, it is expected that over many sets of data the daily variation would be less with shrunk weights than with full weights. It should be pointed out that during the seasons when these weights were taken (late fall and spring) there are extreme changes in weather conditions from day to day in Nebraska.

Single vs. Three-day Average Weights. The procedure used to estimate the separate components of variance assumes the expected variation for days, animals and residual is the same for each of the three days. Bean (1946) found a slight increase in the variation of lot means on each succeeding day in swine weighed for three consecutive days and concluded the weighing operation introduced variation. Baker *et al.* (1947) also

found a slight increase when weighing suckling calves. Patterson's (1947) analysis of steer weights indicated no difference for the three days. An analysis of the variation within days of the Fort Robinson bulls indicated no difference in these data. The assumptions stated above appear to be met in these data.

The error variance associated with the single weight of an animal is estimated by the residual component. The residual components of initial shrunk weights were 77–83 for bulls and 58–61 for heifers. The residual components of final shrunk weights were 92–160 for bulls and 60–95 for heifers. The error variance was significantly larger for bulls than for heifers as judged by F tests of the residual components.

Obtaining more than one weight on an animal permits differences in fill to partially cancel each other out. Thus, the residual component can be reduced $1/n$ where n equals the number of weights obtained. In these data, the error variance of shrunk weights associated with fluctuations in fill are reduced from 77–83 to 26–28, 58–61 to 19–20, 92–160 to 31–53 and 60–95 to 20–32 by using the average of three weights.

Where treatments instead of animals are compared as in feeding trials, the appropriate experimental error is $(\sigma^2_a+\sigma^2/n)\ /m$ where $\sigma^2=$ residual variance, $\sigma^2_a=$ animal variance, $n=$ number of weights and $m=$ the number of animals on a treatment. Using the average of three weights reduces the residual variance but does not reduce animal variance. As an example, the variation in shrunk final weights of Lincoln bulls were $\sigma^2_a=6805$, and $\sigma^2=92$. The variance of single weights equals $6897/m$ and that of 3-day weights equals $6836/m$. The reduction in variation by using a 3-day average instead of a single weight was 0.9%. Similar reductions in the other groups of data shown in table 1 were 0.9, 2 and 1%.

Gain instead of initial or final weight is usually the criterion compared. The variance of gain is the variance of the difference between initial and final weight. If the fluctuations in fill of initial weights are independent of the fluctuations in fill of final weights the residual variation for gain is the sum of the initial and final residual components. Thus, for the Lincoln bulls (shrunk basis), the residual component for gain equals 175 while that of the Fort Robinson bulls (shrunk basis) equals 237. The components of variation due to differences among animals, as determined by an analysis of the gain data, were 2304 for the Lincoln bulls and 1233 for the Fort Robinson bulls.

The residual variation of gains can be reduced by using 3-day average weights from 175 to 58 and 237 to 79, a reduction of 67%. The experimental error when testing for differences between treatments of animals would be 2479 and 1470 for gains based on single weights. Basing gain on the average of three initial and final weights reduces the variation to 2362 and 1312, respectively. A reduction of 5 and 11% in the experimental error for gains.

The errors of weights and gains discussed so far involved animals

weighed on the same set of days. The estimates of variation due to days ranged from 10 to 135 in the shrunk-weight data averaging 43. Where comparisons involve different sets of weigh days, the component for days should be added to the residual and animal components for estimating experimental error. When animals are weighed on more than one day and the average is used, the variation is reduced by $1/n$, where n equals the number of weights taken and if experimental errors are not correlated.

Influence of Size and Age on Accuracy. As animals grow larger the digestive tract increases in capacity and offers opportunity for larger fluctuations in fill. In these data, the residual variation increased with the age of the animal. The increased variation was not proportional to increased weight. This is shown quite strikingly by comparing the Lincoln and Fort Robinson data for shrunk weights. In the Lincoln data, the residual variation increased 11 and 3% for the bulls and heifers and their weights increased 93 and 83%. At Fort Robinson, the residual variation of bulls and heifers increased 108 and 56%, respectively, while their weights increased 46 and 36%. The Lincoln cattle were on a growing-fattening ration, and the Fort Robinson cattle were fed roughage only. Factors of management, the disposition of cattle, ration and differences in body capacity are apparently more important in determining residual variation than difference in weight alone.

Application of Results

Full weights vs. shrunk weights. The results of this study indicate that shrinking cattle for a period of 6 to 12 hours decreased the fluctuations in fill slightly for weanling calves and to a much greater extent among yearling cattle. The variation between animals was also reduced when the cattle were shrunk, presumably because they carried less fill. The use of shrunk weights could be more important where comparisons are made between groups of animals fed different rations that may influence the amount of fill the animals contain. Shrinking cattle for a 6- to 12-hour period is recommended for experimental studies whenever it is practical to do so, particularly where cattle are a year old or more.

Single vs. three-weight average weights. In record of performance programs the weights or gains of animals are compared. The source of error of these comparisons is primarily due to fluctuations in fill. The use of averages of repeated weights is effective in reducing this type of error. The choice of one or more weights would depend on how accurate a comparison is needed. For example, the error of comparison for gains on bulls at Lincoln weighed on the same days was $2(83+92)=350$ (the variance of a difference), with a standard deviation of 18.7 lb. For a particular comparison to be judged as significant, at the 5% level of probability, the difference in gains should be greater than 36 lb. on the basis of single weights. Using the average of three weights, the standard deviation of the difference is 10.7 lb. and 21 lb. or more would be judged as a significant difference.

Where two or three weights are taken they should be separated by several days and not taken on consecutive days. No evidence is presented in this study to justify this recommendation. However, evidence was presented by Baker and Guilbert (1942) on the correlations between deviations in weight for consecutive days.

The use of single or 3-day weights for comparing average weights of groups of animals as in feeding trials presents a different situation. The fluctuations in fill have an opportunity to cancel out even on single-day weights. Where animal differences are as large as in these data, compared to the residual variation, the degree of precision is attained more easily by adjusting the number of animals per lot to the precision required. The practice of using a set number of animals per lot for all experimental trials without regard to the accuracy needed seems wasteful of animals for certain experiments and inadequate for others. There are sufficient data at almost any experiment station to yield reasonable estimates of the variation animal weights or gains for different types of rations and these can be used in planning more efficient use of animals for future experiments.

Summary

Analysis of the three initial and three final weights of 582 calves for full and shrunk conditions was presented. Shrunk weights were influenced less by fluctuations in fill and were considered a more accurate measure of weights and gains. The use of 3-day average weights was effective in reducing fluctuations in fill. The average of three weights or at least two weights is recommended for use where comparisons of individuals are concerned. Animal differences were large in comparison to residual variation. Adjusting the number of animals to obtain the accuracy needed was the most effective way of reducing variation for comparisons of groups of animals.

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SOME FACTORS AFFECTING LITTER SIZE AND FETAL WEIGHT IN PUREBRED AND RECIPROCAL-CROSS MATINGS OF CHESTER WHITE AND POLAND CHINA SWINE [1]

L. N. Baker, A. B. Chapman, R. H. Grummer and L. E. Casida

University of Wisconsin, Madison

THE incidence of prenatal mortality at successive stages of gestation in swine is not known and little appears to be understood of the relationships among the various factors in the maternal environment affecting prenatal survival and prenatal growth. The following study deals with factors affecting litter size and fetal development at two stages of gestation (25 day and 70 day) in matings of purebred Chester White (CW), purebred Poland China (PC) and their reciprocal crosses (CW-PC and PC-CW).

Materials and Methods

All gilts and boars used were from the Experiment Station herd. They were handled uniformly and hand-fed liberally.

The gilts in each breeding group and slaughter stage are classified by the condition of their reproductive organs in table 1.

Three CW and three PC boars of known fertility were used in the study. Each boar was mated to 1–4 potentially fertile gilts in each of the groups to which his breed was to be mated. As a result, each boar sired one or more litters in each such group.

All the gilts classified as abnormal at slaughter showed a bilateral salpingitis. These were discarded from the study. One other gilt showed a unilateral salpingitis. Her total number of corpora lutea was considered in the ovulation study, but for the study of embryo survival she was considered as having produced only the number of ova arising from the ovary on the unaffected side. She was not considered in the studies on litter size. Data on the number of corpora lutea in the ovaries of one other pregnant gilt also were lost in processing. Consequently, she was not included in the study of ovulation or survival.

The numbers of corpora lutea were not available for any of the gilts that proved to be nonpregnant. Most of them had returned to heat and the corpora lutea had regressed before slaughter. Embryo survival in these gilts was considered as zero, but the relationship of survival to the number of ovulations could not be studied in them. Hence, the numbers of ovulations in the analysis were limited to the pregnant gilts.

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All gilts were bred at the first heat after reaching 9 months of age, and all were slaughtered in the course of the experiment. The reproductive organs from those that returned to heat were examined for possible anatomical conditions which would prevent fertilization. The gilts which conceived were slaughtered at the scheduled stage of pregnancy and their reproductive organs removed for dissection. The number of corpora lutea in

TABLE 1. NUMBERS OF GILTS CLASSIFIED BY BREEDING GROUP, CONDITION OF THEIR REPRODUCTIVE ORGANS AND INTERVAL AFTER BREEDING TO SLAUGHTER

Breed		Days after breeding when slaughtered	No. gilts at slaughter		
Boar	Gilt		Abnormal	Normal-not pregnant	Normal-pregnant
CW	CW	25	1	1	8
		70	0	1	5
	PC	25	1	0	5
		70	0	1	7
PC	CW	25	2	0	5
		70	0	1	6
	PC	25	0	2	6
		70	0	0	7

each ovary was recorded. Each fetus with its surrounding membranes and fluids (amniotic and allantoic) was removed separately and the weights of the fetuses, membranes and fluids were recorded.

The effects of breed of sire, breed of dam, and kind of mating for each stage of pregnancy were studied by analysis of variance using the method of unweighted means because of the inequalities in sub-class numbers (Snedecor, 1956). The interrelations of the various fetal and litter characters were studied by means of standard partial regressions calculated on a within-breeding-group basis.

Results and Discussion

Effects of Breed and Kind of Mating on Factors Affecting Litter Size and Fetal Development

Ovulation Rate. Breed had a significant effect on the number of corpora lutea in the pregnant gilts. CW gilts exceeded the PC gilts by an average of 1.4 (simple average of corpora lutea for all pregnant gilts within the breed: CW, 13.3; PC, 11.9; $P<0.05$). Differences in favor of the CW

ranging from 2.2 to 3.6 have been noted by others at this station (Robertson *et al.*, 1951a, b; Self *et al.*, 1955).

Embryo Survival Rate. The percent embryo survival (number of fetuses divided by number of corpora lutea times 100; nonpregnant gilts considered as having zero percent embryo survival) did not vary significantly for breed of sire, breed of dam, kind of mating, or stage of pregnancy. However, there appeared to be a tendency (statistically insignificant)

TABLE 2. NUMBERS OF CORPORA LUTEA AND FETUSES AND AVERAGE WEIGHTS OF FETUSES AND THEIR MEMBRANES AND FLUIDS

Stage	Breed of		No.[a] C.L.	No. fetuses per litter	Percent[b] survival all gilts	Average wt. per litter		
	Sire of fetuses	Dam of fetuses				Fetuses	Membranes	Fluids
25-day	CW	CW	14.2	9.8	62	628[c]	10.7[d]	.109[d]
		PC	12.8	10.2	82	502	8.6	80
	PC	CW	12.4	7.8	64	477	7.9	85
		PC	11.0	9.8	72	571	8.3	99
	Average		12.6	9.4	70	544	8.9	93
70-day	CW	CW	12.8	6.2	36	266[d]	273[d]	343[d]
		PC	11.6	5.7	40	252	278	329
	PC	CW	13.2	9.4	67	243	216	398
		PC	12.0	7.1	64	255	266	384
	Average		12.4	7.1	52	254	258	363

[a] In pregnant gilts.
[b] Survival percentage for each gilt transformed to an angle for averaging; these averages were retransformed into percentages for tabulation.
[c] Milligrams.
[d] Grams.

toward interaction of breed of sire with stage. The mean percentage-survivals at 25 and 70 days were 62 and 36, and 82 and 40 for the CW boars on CW and PC gilts, respectively; whereas, corresponding values for the PC boars were 64 and 67, and 72 and 64 (table 2).

The probability of survival for an individual embryo was found to differ markedly from one dam to another at both the 25- and 70-day stages (ratios of number of corpora lutea to number of fetuses for different gilts were heterogeneous—$P<0.01$). Estimates of the intragilt correlations for the gilts that were pregnant (ratio of the non-binomial variance to the total variance within breeding groups, Cochran, 1943) were 0.75 at the 25th day and 0.73 at the 70th day. Thus, it appears that different gilts

express unequal ability to maintain living embryos thereby reflecting their individuality—genetic and/or environmental.

Litter Size. Litter size was not found to differ significantly between the two breeds of gilts at either of the two stages studied (table 2). The average litter size at the 25-day stage, however, was 9.4 and at the 70-day stage 7.1—a significant difference of 2.3 fetuses. Self *et al.* (1955) reported that CW gilts had larger litters than PC gilts at 25-days in one year and that no breed-of-gilt difference was apparent in another. Their gilts, however, were approximately 2 months younger at breeding than the present gilts.

Litter sizes in CW-sired litters at the 25th and 70th days of pregnancy were 10.0 and 6.0 (as calculated from table 2), whereas in the PC-sired litters they were 8.8 to 8.3, respectively. This is a significant interaction and is consistent with the possible interaction noted above between breed of sire and stage of pregnancy for percentage embryo survival analyzed over all normal gilts. This suggests a breed-of-sire difference in embryo mortality between the 25-day and 70-day stages. Such a result could be due to partial sterility factors carried by the CW males. A defect in a part of the spermatogenic meioses which became lethal between 25 and 70 days of gestation is implied. This appears to have occurred in laboratory mammals (Tyler and Chapman, 1948; Snell, 1933) and has been postulated for cattle (Knudsen, 1956) and for man (Sonneborn, 1956).

Fetus Weight and Associated Characters. The effects of breed of sire and breed of dam on fetus, membrane and fluid weight were insignificant. Kind of mating was found to have a significant effect on fetus weight at the 25th day. Purebred fetuses were heavier than crossbreds (table 2). A similar, but insignificant, trend was found at 70-days in these data. Robertson *et al.* (1951b) reported that the average length of fetuses out of CW gilts was significantly greater than the average for fetuses out of PC gilts. Such a breed-of-dam difference was not found for weight of fetus in the present data.

The lesser weights of the crossbred fetuses than of the purebreds has not been widely demonstrated. It is pronounced at the 25-day stage only. The limited time at which it appears to be demonstrable would make its detection unlikely. Inhibitory gene interaction within the embryo may be operative during an early phase of development or inhibitory maternal effect on "foreign" genes in the embryo might occur during the earlier course of development.

Both the breed-of-sire effect on litter size noted above and the kind-of-mating effect upon embryo growth need further study in swine. These effects were not anticipated in this study; hence, no *a priori* covering hypotheses were under test.

Interrelationships of Factors Affecting Litter Size and Fetus Weight

Ovulation and Survival Rate. Litter size is determined primarily by ovulation rate and embryo survival. The over-all estimate of fertilization rate

in gilts by boars of established fertility is about 95% (Robertson *et al.*, 1951a, b; Squiers *et al.*, 1952; Self *et al.*, 1955).

Survival, up to the 25th day including presumed cases of total embryo loss and averaged over all breeding groups, was 70% (table 2). Self *et al.* (1955), reported survival rates of 44 and 59% up to this same stage for two successive years. However, their study involved some feeding practices which in themselves increased embryonic death. Survival to the 70th day in the present study was 52%. Survival based on pregnant sows only and not including cases of presumed total embryonic death was 79% at 25 days and 62% at 70 days. A similar estimate by Squiers *et al.* (1952) to the 25th day was 64% survival. They also arrived at an estimate of survival to term of 54% by comparing the litter size of gilts allowed to go to term with the known ovulation rate of similar gilts slaughtered during pregnancy. Hammond (1921), Corner (1923) and Warwick (1928) have estimated embryo survival to term as being approximately 70%. The greater magnitude of this latter value as compared to the above mentioned estimates is thought to be due to the fact that the latter estimate was based on packing house observations, i.e., data which involved both gilts and sows or only sows and which did not include cases of total embryonic death. Thus, it appears that the greatest portion of embryonic mortality occurs prior to the 25-day stage and that very little occurs after the 70-day stage.

TABLE 3. RELATIONSHIP OF LITTER SIZE (Y) WITH OVULATION RATE (X_1) AND EMBRYO SURVIVAL RATE (X_2) AT THE 25 AND 70 DAY STAGES

Item	Stage	
	25-day	70-day
d/f	19	19
r_{Y1}	0.37	0.09
r_{Y2}	0.90	0.94
r_{12}	−0.08	−0.27
$b'_{Y1.2}$	0.48	0.36
$b'_{Y2.1}$	0.93	1.03

Standard partial regression coefficients within breeding groups have been used to indicate the relative influence which ovulation rate and embryo survival had on litter size (estimated for pregnant gilts only) at the two stages of pregnancy (table 3). The data were transformed to logarithms to provide an additive scale. Embryo survival rate was apparently the more important of the two factors at both stages (25-day, 0.93 vs. 0.48; 70 day, 1.03 vs. 0.36). These results are comparable with those calculated by Squiers *et al.* (1952). It the gilts which were bred but were nonpregnant at their designated slaughter stage had been included in the estimates the importance of embryo survival would have been accentuated

as compared to ovulation rate. They were not included, however, because the numbers of corpora lutea for them were not known.

Fetus Weight with Other Fetal Characters. The linear association of the fetus weight with membrane and fluid weight is shown in table 4. The correlations between membrane weight and fluid weight were 0.70 at the 25th day and 0.30 at the 70th day. The data were examined on the assumption of the dependence of fetus weight on membrane and fluid weights. The standard partial regressions of fetus weight upon fluid weight and upon embryo weight differed significantly from each other at the

TABLE 4. RELATIONSHIP OF LITTER-AVERAGE FETUS WEIGHT (Y) WITH LITTER AVERAGE MEMBRANE (X_1) AND FLUID WEIGHT (X_2) AT THE 25- AND 70-DAY STAGES

Item	Stage	
	25-day	70-day
d/f	19	20
r_{Y1}	0.35	0.65
r_{Y2}	0.73	0.44
r_{12}	0.70	0.30
$b'_{Y1.2}$	—0.32	0.57
$b'_{Y2.1}$	0.96	0.27
$R_{Y(21)}$	0.77	0.70

25-day stage and each of these regressions differed from the comparable regressions at the 70-day stage. Membrane weight had a negative direct effect on fetus weight at the 25th day (—0.32) as compared to a positive effect of fluid weight (0.96). Both effects were positive at 70 days; 0.57 for membranes and 0.27 for fluids.

The observed relationships at 25 days are consistent with the concept that variations in fetal development are partially dependent on variations in membrane permeability. The quantity of fetal fluids might be considered as one indicator of that permeability. The negative relationship between fetus weight and membrane weight with fluid weight held constant can point to the thickness of the membrane acting as a barrier to transport whereas the greater surface area associated with increased fluids enhances transport. By the 70-day stage, the situation has just about reversed and the effect of membranes on fetus weight is reflected much less in fluid weight. Variation in the gross vascular supply of the chorion (a major constituent of the weight of the chorion) perhaps becomes more important at the latter stage as contrasted to variation in permeability at the earlier age.

Fetus Weight with Number of Fetuses. The number of young in a litter is well recognized as affecting birth weight in various species. The correlations of litter size with litter-average fetus weight were 0.30 at

25 days and —0.06 at 70 days. Neither of these was statistically significant but there was a suggestion that the relationship might be positive early in pregnancy and change to negative as gestation advances. Possibly, factors which favor high ovulation rate also favor rapid fetal growth early in pregnancy; whereas, later in pregnancy, competition and crowding in larger litters tend to retard fetal growth.

The depressing effects of large litter size on embryonic growth have been attributed in part to "general" factors and in part to "local" (Eckstein and McKeown, 1955; Eckstein, McKeown and Record, 1955).

TABLE 5. RELATIONSHIP OF AVERAGE FETUS WEIGHT IN THE UTERINE HORN (Y) WITH LITTER SIZE (X_1) AND NUMBER OF FETUSES IN THE UTERINE HORN (X_2)

Item	Stage	
	25-day	70-day
d/f	43	41
r_{Y1}	0.28	—0.02
r_{Y2}	0.27	—0.24
r_{12}	0.93	0.72
$b'_{Y1.2}$	0.23	0.31
$b'_{Y2.1}$	0.05	—0.45
$R_{Y(12)}$	0.28	0.32

Competition for the general nutrient supply can illustrate the former and competition for space in a uterine horn the latter

Litter size has been used as an indicator of the intensity of "general"-competition within the dam and number of fetuses in the uterine horn as an indicator of local competition. These two values are positively correlated in swine, r=0.93 and 0.72 at the 25- and 70-day stages, respectively (table 5). Consequently, correlations between number of fetuses in the uterine horn and fetal weight might be expected. These were 0.27 and —0.24 at the successive stages.

Standard partial regressions were used to study the relative importance of number in the litter and number in the horn (table 5), each independent of the other, in determining the average weight of fetuses in the uterine horn. At the 25-day stage, neither was statistically significant and both were small and positive, 0.23 and 0.05, respectively. A marked change had occurred by the 70-day stage. The regression on the number of fetuses in the horn was now significant and negative and differed (P=0.01) from the regression on litter size (—0.45 vs 0.31).

Perhaps the simplest mechanism to explain the different effects of increased numbers of pigs in the uterine horn as compared to increased litter sizes upon fetal weight is interference with elaboration of the placenta. This might come about from mechanical crowding or from com-

petition for nutrients in the local vascular supply. It was assumed therefore (table 6) that both litter size and number of fetuses per horn affected fetal weight by means of the variation in membrane weight to the full extent of their association with membrane weight. The existence of remaining direct effects would then be evidence of other channels of operation.

Direct effects were statistically insignificant for both litter size and number of fetuses per horn at both the 25-day and 70-day stages. Both

TABLE 6. RELATIONSHIP OF AVERAGE WEIGHT OF FETUSES IN A UTERINE HORN (Y) WITH LITTER SIZE (X_1), NUMBER OF FETUSES IN A UTERINE HORN (X_2) AND AVERAGE WEIGHT OF FETAL MEMBRANES IN A UTERINE HORN (X_3)

Item	Stage	
	25-day	70-day
d/f	43	41
r_{Y1}	0.28	−0.02
r_{Y2}	0.27	−0.24
r_{Y3}	0.37	0.65
r_{13}	−0.52	−0.10
r_{23}	−0.50	−0.21
r_{12}	0.93	0.72
$b'_{31.2}$	−0.37	0.09
$b'_{32.1}$	−0.16	−0.28
$b'_{Y1.23}$	0.50	0.25
$b'_{Y2.13}$	0.16	−0.28
$b'_{Y3.12}$	0.70	0.61
$R_{3(12)}$	0.52	0.22
$R_{Y(123)}$	0.66	0.68

were positive at 25 days, 0.50 and 0.16 (table 6). Whereas, the indirect effects by way of membrane at 25 days were both negative ($-0.37\times0.70=-0.26$ and $-0.16\times0.70=-0.11$). The direct effect at 70 days was positive for litter size, 0.25, but it had become negative, −0.28, for number of fetuses in the horn suggesting a depressing effect of number of fetuses in the horn mediated by some path other than gross mass of the placenta.

The indirect effects of litter size were quite small at 70 days ($0.09\times0.61=0.05$), and of number of fetuses in the horn it was essentially the same as at 25 days, ($-0.28\times0.61=-0.17$). The regression of fetal weight on membrane weight with both pig numbers held constant was much the same at the two stages, 0.70 and 0.61.

Summary

Factors affecting litter size and fetal development as determined at 25 and 70 days of gestation were studied in matings of 55 gilts and 6

boars including purebred Chester White (CW), purebred Poland China (PC) and their reciprocal crosses (CW-PC and PC-CW).

The two breeds of gilts differed in ovulation rate (CW 13.3; PC 11.9; $P<0.05$). Litters sired by CW boars showed a greater difference in number of pigs between the 25th and 70th days of gestation than did litters sired by PC boars. When averaged over both purebred and crossbred matings, the difference was between 10.0 and 6.0 for CW sired litters on the respective days and between 8.8 and 8.3 for PC. A difference between these two breeds of sires in their embryo mortality is suggested.

Purebred fetuses were significantly heavier than crossbred fetuses at the 25th day, and a similar but insignificant trend was found at the 70th day.

Approximately three-fourths of the variance in the probabilities of embryo survival in different pregnant gilts was nonbinomial in character indicating individual differences in the maternal contribution to survival, genetic plus environmental. Embryo survival rate appeared to have greater influence than ovulation rate in determining litter size.

Membrane weight in terms of standard partial regression was significantly less important than fluid weight in the determination of fetus weight at the 25th day. It became significantly more important by the 70th day and fluid weight significantly less.

Fetus weight was positively but insignificantly associated with both number of fetuses in the litter and numbers of fetuses in the uterine horn at the 25th day. By the 70th day, the standard partial regression on numbers of fetuses in the horn was significant and negative and differed significantly from the standard partial regression on litter size (—0.45 vs. 0.31). There was a trend between the 25th and 70th days toward the development of an effect of number of fetuses in the uterine horn upon fetus weight which could not be accounted for by their association through membrane weight.

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THE RELATIONSHIP OF THE DAM'S FEEDING REGIMEN, BREED, AND INDIVIDUALITY TO FETAL DEVELOPMENT IN SWINE [1]

D. P. WALDORF,[2] H. G. SPIES, D. R. ZIMMERMAN, H. L. SELF, A. B. CHAPMAN AND L. E. CASIDA

University of Wisconsin, Madison

THE ease with which the quantity of nutrients fed to swine may be altered without producing marked physiological disturbances makes it possible to study the effects of complex feeding management systems on reproductive performance. Prenatal growth, because of the association between birth weight and postnatal growth and viability (Kuhlman, 1928; Vestal, 1936), merits study relative to its response to feeding management. McKenzie (1928) found that the birth weights of pigs from dams grown and maintained on a restricted feed intake were not markedly depressed as compared to those on a liberal intake, but the size of the litters from the females on the low plane of nutrition was reduced. Vestal (1936), using two quite different planes of nutrition fed only during the gestation period, could not produce differences in the average birth weight or the size of the litters from sows and gilts.

Breed has been indicated as a cause of differences in birth weights (Carmichael and Rice, 1920). Differences in maternal weight gain during gestation (Donald and Fleming, 1938), age of dam at first litter and litter size (Carmichael and Rice, 1920; Kuhlman, 1928; Lush *et al.*, 1934; Donald and Fleming, 1938) have been shown to have an effect on the birth weights of pigs. The effect of these individual differences, and others, have been examined in both sows and gilts by Waldorf *et al.* (1957).

The purpose of this study is to examine differences in fetal growth arising from, (a) breed of dam, (b) the feeding regimen to which the dam has been subjected, and (c) certain of the dam's growth and litter characteristics—to gain some understanding of how these previously noted results might have arisen. Ultimately, the goal of the investigation, of which this study is a part, is to determine a sequence of feed levels that will yield larger litters with satisfactory birth and weaning weights.

Materials and Methods

General. Poland China (PC) and Chester White (CW) gilts from the University of Wisconsin purebred herd were used in three trials, each

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[2] Ralston-Purina fellow; deceased October 29, 1957.

trial starting with some of the animals being full-fed and some being limited-fed. The gilts of each breed were allocated at random to the various feed groups and started on experiment at 70 to 115 days of age. Table 1 shows the number of gilts completing the experiment per subgroup. The rations used in these trials are shown in table 2.

Daily heat checks with vasectomized boars were started when the first gilt in a trial reached 150 days of age, and they were continued until

TABLE 1. THE EXPERIMENTAL PLAN SHOWING THE NUMBER OF GILTS HAVING LITTERS IN THE DIFFERENT SUBGROUPS

Feed level sequence [a]	Stage of gestation at end of experiment	Trial I		Trial II		Trial III	
		CW	PC	CW	PC	CW	PC
FFFF [b]	25 days			5	4	10 [f]	11 [f]
	70 days	4	4				
	105 days	5	5			5	6
	Term			4	5		
LFLL [c]	25 days			5	5		
	70 days	3	5				
	105 days	1	3				
	Term			3	3		
LFLF [d]	105 days	3	4				
LLLL [e]	25 days					12 [f]	13 [f]
	70 days	4	4				
	105 days	4	4			6	5

[a] F indicates full-feeding; L indicates limited-feeding.
[b] Full-fed throughout the experiment.
[c] Limited-fed throughout the experiment except for a "flushing" period of two or three weeks just before breeding.
[d] Limited-fed and then flushed; again limited-fed following breeding until 70 days of gestation when changed to full-feeding until the end of the experiment.
[e] Limited-fed throughout the experiment.
[f] Approximately one-half of these gilts received progesterone. See text for details.

all gilts were past their fiftieth day of gestation or were 300 days old without having been in heat. Any gilts to be bred at a particular estrual period were bred once on the first day of that period. Animals returning to heat after breeding were slaughtered, except as noted in trial I. As individuals reached 210 days of age (230 days in trial II) their weight and back-fat thickness were recorded. All live back-fat measurements were obtained as a single measurement at about the seventh rib, 1½ to 2 in. from the midline (Hazel and Kline, 1952). The weight and back-fat thickness of each gilt were also obtained just before slaughter. Carcass weights were obtained for all gilts; the carcass length (front of the aitch bone to the front of the first rib) of most gilts was also recorded in trial III.

A corpora lutea count was made for each gilt at slaughter. The data

TABLE 2. RATIONS USED IN THE DIFFERENT TRIALS

Item	Trial I		Trial II			Trial III				
Calc. crude protein, % [a]	19.0	13.7	14.3	12.4	17.0	16.0	15.8	14.2	16.0	14.0
Calc. fiber, % [a]	4.7	4.6	11.4	15.8	3.9	13.3	14.2	18.0	5.9	5.8
Constituent, lb.										
Corn	525	615	425	300	640	250	220	115	535	585
Oats	225	260	160	160	150	250	250	250	250	250
Soybean meal	100	40	115	90	150	100	100	90	80	55
Tankage	90	40	50	50	50	...	...	...	...	...
Meat scraps	...	...	...	...	...	100	100	90	80	55
Linseed meal	52.5	30	...	...	...	...	...	...	...	...
Alfalfa meal	...	...	...	...	...	50	50	50	50	50
Corn cobs	...	...	250	400	...	245	275	400	...	...
Trace mineralized salt	5	5	5	5	5	5	5	5	5	5
Ground limestone	...	...	5	5	5	...	...	...	...	...
Zinc sulphate·$7H_2O$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Antibiotic mixture	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vit. A supplement	...	5	...	...	...	...	...	...	...	...
Irradiated yeast	...	0.25	0.25	0.25	0.25	...	...	...	...	...
Total weight	1001.5	998.25	1018.25	1018.25	1008.25	1003	1003	1003	1003	1003

[a] Morrison (1950).

on the litters were obtained as follows: (1) The 25 day embryos and their membranes were removed from the uterus, separated, drained, and weighed in uterine horn groups. (2) The 70- and 105-day fetuses were prepared similarly; the fetuses and membranes were weighed individually, and the sex of the fetus was recorded. (3) The farrowed pigs were weighed individually shortly after being farrowed, and their sex was recorded.

Trial I. The 71 gilts (36 CW and 35 PC) used in this trial were born between April 29, 1955, and May 20, 1955, and put on experiment as they reached 70 days of age when their average weight was 41.7 lb. Gilts being limited-fed at any time during the experiment were given the same ration as the full-fed (self-fed) gilts, but at about two-thirds the level estimated to be eaten concurrently by the full-fed animals (generally 65–80% of the consumption of the self-fed gilts during the previous two weeks). The feed intake of the limited-fed gilts was restricted by hand-feeding them twice daily with the calculated quantity of ration. The initial protein level fed to all gilts was 19% (table 2); this was reduced to 13.7% when the group averaged 154 days of age.

At puberty, a part of the limited-fed gilts were put in lots where they could be full-fed until second heat (flushed). All other gilts were changed to other lots at puberty without a change in feed level. This was done so that all gilts would experience a change in pen. At second heat, all gilts were bred and returned to their original lots, the "flushed" gilts being put back on limited feeding.

Eight purebred, spring-born Duroc boars were used for breeding the experimental gilts. Successive random orders of these eight boars were made up; the order in which gilts came in heat for breeding was matched with this overall sequence of boars to obtain the individual matings. Flushed gilts returning to heat after breeding were slaughtered; other gilts returning to heat were rebred once to the same boar. Some of the gilts on each feed sequence were slaughtered at 70 days; the rest were slaughtered at 105 days. The gilts remaining in the flushed group after 70 days were divided at random into two groups, one being continued on limited feeding and one being changed to full-feeding (table 1).

Trial II. Twenty-three gilts (12 CW and 11 PC) born between September 29, 1955 and October 28, 1955, were put on experiment at 75 days of age at an average weight of 41.0 lb. Another group of 24 gilts (12 of each breed) born between August 19, 1955 and September 5, 1955, was put on experiment at 115 days of age at an average weight of 94.5 lb. These two groups of gilts were treated similarly but kept in separate pens.

Both of the rations used were self-fed; however, one contained 40% corn cobs. It was intended that the protein levels in the rations would be about equal; however, an unnoticed error in feed ordering prevented this, the resulting levels being 12.4% and 17.0% in the low-fiber and high-fiber rations, respectively (table 2). The gilts that were put on experiment

at 75 days of age and fed the high-fiber ration failed to gain at an adequate level (at approximately two-thirds that of their low-fiber controls). Approximately 2 weeks after they were put on experiment, the level of corn cobs in their ration was reduced to 25% and held there for 4 weeks (March 9 to April 7); they were then returned to their original ration.

The gilts were bred at their second heat after reaching 190 days of age. Three Chester White and three Poland China boars were used for breeding in this trial. Each of the three gilts of a breed in each subgroup was bred at random to a different boar of the other breed.

Limited-fed gilts were flushed by putting them on the low-fiber ration for approximately the last two weeks of their first complete estrual cycle after 190 days of age. They were bred and returned to the high-fiber ration at the end of this cycle. When the animals being allowed to farrow reached 111 days of gestation, their back-fat thickness was obtained. They were weighed shortly after farrowing was completed.

Trial III. Seventy-four gilts (36 CW and 38 PC) born between May 4, and May 25, 1956, were allotted to the treatment groups shown in table 1 when they reached 90 days of age. The average weight of these gilts was 65.6 lb. The limited-fed animals were started on a ration containing 24.5% corn cobs; however, this was increased to 27.5% cobs in one month and held there until the group averaged 154 days of age. At this latter time, the level of corn cobs was increased to 40%, and the protein level was dropped from 15.8% to 14.2%. The protein level of the low-fiber ration was similarly changed (table 2).

The matings used in this trial were made up by the method used in trial I. Since there were two breeds of boars (CW and PC) to be used in making reciprocal crosses, the random orders of boars were arranged within breeds.

A random half of the gilts in each breed-feed level subgroup to be slaughtered at 25 days of gestation was given progesterone from the tenth day of gestation until slaughter in connection with another experiment not reported here. Two dosage levels (1.0 mg./lb. body wt./day and 0.4 mg./lb. body wt./day) were given subcutaneously in corn oil daily. Data from these progesterone treated gilts were used for the multiple regression analyses; however, they were not used in this study for comparisons between subgroups.

Statistical Procedures. All analyses are based on weights of normal fetuses (showing no gross postmortem changes or anatomical abnormalities), these weights being adjusted for any significant differences due to sex. The path coefficient (standard partial regression) analyses were done as in Waldorf *et al.* (1957). Where information from two trials was used for path coefficient analysis, the correlations used were based on pooled sums of squares and crossproducts.

TABLE 3. MATERNAL GROWTH BY BREED AND FEEDING REGIMEN

Subgroup[a]		210 days of age[b]		25 days of gestation				70 days of gestation		105 days of gestation				Term	
		Weight	Back-fat	Carcass weight	Back-fat	Carcass length		Carcass weight	Back-fat	Carcass weight	Back-fat	Carcass length		Post-partum weight	Back-fat
		lb.	in.	lb.	in.	in.	in.[c]	lb.	in.	lb.	in.	in.	in.[c]	lb.	in.
Trial I:															
FFFF	CW	228.1	1.9					250.0	2.4	250.2	2.2				
	PC	261.7	2.0					280.0	2.5	305.8	2.7				
LFLF	CW	190.7	1.5							247.0	2.2				
	PC	189.0	1.3							238.2	1.9				
LFLL	CW	213.0	1.7					187.3	1.7	240.0	1.9				
	PC	193.7	1.4					167.6	1.9	205.0	1.5				
LLLL	CW	201.1	1.5					206.2	1.6	236.5	1.9				
	PC	187.5	1.4					184.5	1.8	212.0	1.9				
Trial II:															
FFFF	CW	273.0	2.3	252.6	2.6									356.5	2.4
	PC	287.2	2.4	262.8	2.6									382.6	2.6
LFLL	CW	225.7	1.7	177.4	2.0									358.0	2.4
	PC	240.4	1.7	199.2	1.8									349.0	2.2
Trial III:															
FFFF	CW	231.4	2.2	242.3	2.5	31.3	31.0			297.0	2.8	33.5	33.4		
	PC	265.7	2.0	245.6	2.1	30.7	31.0			342.7	3.0	33.8	34.0		
LLLL	CW	183.4	1.5	161.9	1.6	30.3	30.6			209.8	2.0	32.6	32.5		
	PC	181.5	1.2	165.7	1.3	29.6	29.7			208.8	1.8	31.9	31.4		

[a] See footnotes, table 1.
[b] 230 days of age in trial II.
[c] Adjusted for age using the regression within subgroups of carcass length on age.

Results

Maternal Growth—as Affected by Feeding Regimen and Breed. The various weights and measurements taken on the live animals and on the carcasses indicate that the growth of the limited-fed (LFLF, LFLL, LLLL) gilts was slightly retarded compared to that of the full-fed (FFFF) gilts (table 3). The weights and back-fat thicknesses of the limited-fed gilts at both ages (210 and 230 days) were 75–85% and 65–75%, respectively, of those of the full-fed gilts. At slaughter, comparable differences in carcass weight and back-fat thickness were found. There did not seem to be any consistent breed differences in these groups of animals with regard to these characters.

The data obtained on carcass length of the gilts in trial III indicated that differences in skeletal growth were produced by the feed levels (table 3). At both stages, the limited-fed gilts had shorter carcasses than the full-fed gilts (25-day gilts, $P<0.05$; 105-day gilts, $P<.01$); there were no clear differences due to breed. Adjustment of these carcass lengths for the age of the gilt on the basis of the regression within subgroups affected the differences between subgroups slightly, but the feed effects retained their original statistical significance. In the 105-day gilts, the breed-feed level interaction became significant, possibly indicating that the latter skeletal growth of the Poland China gilts was affected more by differences in feed level than was that of the Chester White gilts.

Fetal Growth—as Affected by the Maternal Feeding Regimen and Breed. No striking effects of differences in feed level on embryo and fetus weight are apparent in tables 4–7. (All values in these tables have been adjusted for differences in litter size using the regression within subgroups of fetus (membrane) weight on litter size. The negative associations—at 25 days (membrane weight only), 70 days, 105 days, and term—are interpreted as indicating the effects of competition on fetal growth. The meaning of the positive association between embryo weight and litter size found at 25 days is not readily apparent. If it reflects a prefertilization condition in the dam affecting both litter size and fetus weight, there is doubt as to whether the adjustment should have been made. In any case, these adjustments did not alter greatly the differences among subgroups. The relationship of fetus and membrane weights to the maternal feeding regimen at 70 days was tested by comparing FFFF litters with the pooled LFLL and LLLL litters. At 105 days, the comparisons in trial I were made between FFFF and LLLL litters and between LFLF and LFLL litters. None of these individual comparisons is significant. The apparent tendency for full-fed gilts to have heavier pigs at 105 days and term was not significant over the three trials (pooled $P=0.30$). The fetal membranes showed no evidence of being affected by differences in feed level at any stage.

Embryos from Chester White gilts at 25 days of gestation did not

TABLE 4. THE EFFECT OF MATERNAL BREED AND FEEDING REGIMEN ON 25 DAY AVERAGE FETUS AND MEMBRANE WEIGHTS IN GRAMS
(Adjusted for litter size)

Trial	Feed level sequence [a]	Embryo weight			Membrane weight		
		CW	PC	Both	CW	PC	Both
II	FFFF	.386	.460	.423	7.29	4.17	5.73
	LFLL	.512	.490	.502	9.85	7.14	8.50
	Both	.450	.475	.462	8.57	5.66	7.12
III	FFFF	.515	.582	.548	10.62	6.25	8.44
	LLLL	.483	.523	.503	10.09	7.70	8.90
	Both	.499	.552	.526	10.36	6.98	8.67

[a] See footnotes for table 1.

differ significantly in weight from those out of Poland China gilts. At 70 days of gestation, the fetuses from Chester White gilts are heavier than those from Poland China gilts ($P<0.01$). The evidence accumulated over three trials indicates that 105 days and term the fetuses from Chester White gilts are also heavier (pooled $P=0.02$). Breed differences in membrane weight do not follow the same pattern as occurs with the fetuses. At 25 days, the membranes from Chester White gilts are heavier than those from Poland China gilts, significantly so in trial III. This breed difference in membrane weight is highly significant over both trials as determined by combining the probabilities. At 70 days of gestation, the same tendency exists, though it is not significant. By 105 days of gestation, the membranes from Poland China gilts tend to be heavier, significantly so in trial III but not overall.

The Correlation of Maternal Environment and Fetal Growth within Feed and Breed Groups. Our experiments did not demonstrate that fetal growth is affected by a feeding regimen that alters the growth and fatness of the dam. The question that then arises is whether the average growth

TABLE 5. THE EFFECT OF MATERNAL BREED AND FEEDING REGIMEN ON AVERAGE 70 DAY FETUS AND MEMBRANE WEIGHTS IN GRAMS
(Adjusted for litter size)

Trial	Feed level sequence [a]	Fetus weight			Membrane weight		
		CW	PC	Both	CW	PC	Both
I	FFFF	247	211	229	216	227	222
	LFLL	238	203	221	226	218	222
	LLLL	226	174	200	210	166	188
	All three	237	196	217	217	204	211

[a] See footnotes for table 1.

TABLE 6. THE EFFECT OF MATERNAL BREED AND FEEDING REGIMEN ON AVERAGE 105 DAY FETUS AND MEMBRANE WEIGHTS IN GRAMS
(Adjusted for litter size)

Trial	Feed level sequence [a]	Fetus weight			Membrane weight		
		CW	PC	Both	CW	PC	Both
I	FFFF	990	870	930	239	218	229
	LFLF	917	845	881	179	220	200
	LFLL	918	720	819	179	180	179
	LLLL	946	788	867	234	222	228
	All four	943	806	874	208	210	209
III	FFFF	1039	888	963	229	244	236
	LLLL	914	930	922	211	264	237
	Both	976	909	943	220	254	237

[a] See footnotes for table 1.

of the fetuses in the litter is affected by variation in the individual dam's physical development where the environment for the different dams is presumably the same. The effect of variation in the dam's age, carcass weight, and back-fat thickness on litter average fetus and membrane weights was studied by means of path coefficients. The means and standard deviations of these characters are given in table 8. Without presenting the details of this study, it may be said that neither the individual direct effects nor the joint effects of these variables were significant.

Comparing the effects of general and local competition (number of fetuses per litter and per horn, respectively) revealed differences in their relative importance associated with stage of gestation (table 9). At 25 days, the factors conducive to large litters also seem to favor the development of the embryos in the litter ($P_{Y1:2}$=0.27; P=0.12); however, by 105 days the direct effect of litter size on fetus weight has become negligible ($P_{Y1:2}$=−0.03). The influence of local competition seems to become greater as gestation progresses ($P_{Y2:1}$=−0.10; −0.21; −0.47 at 25, 70 and 105 days, respectively), the path from number of pigs per horn to fetus weight being highly significant at 105 days. The joint effect

TABLE 7. THE EFFECT OF MATERNAL BREED AND FEEDING REGIMEN ON AVERAGE PIG BIRTH WEIGHTS IN POUNDS
(Adjusted for litter size)

Trial	Feed level sequence [a]	Breed of dam		
		CW	PC	Both
II	FFFF	2.67	2.58	2.62
	LFLL	3.14	2.59	2.86
	Both	2.90	2.58	2.74

[a] See footnotes for table 1.

TABLE 8. THE MEANS AND STANDARD DEVIATIONS OF CHARACTERS STUDIED BY PATH COEFFICIENT ANALYSIS

Item	Stage of gestation					
	25 days		70 days		105 days	
	Means [a]	S.D.[b]	Means	S.D.	Means	S.D.
Age at slaughter, days	252.4	14.6	279.3	12.8	320.8	11.3
Carcass weight, lb.	213.4	22.9	212.6	25.0	253.2	30.5
Back-fat at slaughter, in.	2.1	0.3	2.0	0.2	2.2	0.3
Litter size	8.9	3.1	7.7	2.2	7.5	2.7
No. fetuses per horn	4.5	1.2	4.1	1.3	3.8	1.4
Horn av. membrane wt., gm.	8.1	3.2	210.6	42.9	222.7	49.8
Horn av. fetus wt., gm.	0.5	0.1	217.3	26.1	912.5	145.6

[a] Unweighted averages of subgroup means.
[b] Based on the average within-subgroup mean squares.

of these two factors is of considerable importance at 105 days of gestation ($R_{Y(12)}=0.50$, $P<0.01$) compared to that at 25 days of 0.19, $P>0.05$. The membranes exhibit this same change with advancing gestation relative to the joint effect of number of fetuses per horn and per litter ($R_{3(12)}=0.18$; 0.26; 0.45, $P<0.01$) at the successive stages.

TABLE 9. THE RELATIONSHIP OF HORN AVERAGE, FETUS (Y) AND MEMBRANE WEIGHT (X_3) TO EACH OTHER AND TO LITTER SIZE (X_1) AND NUMBER OF FETUSES PER HORN (X_2)

Item	Stage of gestation		
	25 days	70 days	105 days
d.f.	112	40	86
r_{13}	—.14	—.26	—.43**
r_{23}	—.18	—.21	—.44**
r_{12}	.84**	.76**	.86**
r_{Y1}	.18	—.12	—.44**
r_{Y2}	.12	—.18	—.50**
r_{Y3}	.66**	.77**	.82**
$P_{31\cdot2}$	.01	—.24	—.20
$P_{32\cdot1}$	—.19	—.03	—.26
$P_{Y1\cdot2}$	.27	.04	—.03
$P_{Y2\cdot1}$	—.10	—.21	—.47**
$P_{Y1\cdot23}$	.26*	.23	.12
$P_{Y2\cdot13}$	.03	—.18	—.27*
$P_{Y3\cdot12}$	.70**	.79**	.75**
$R_{3(12)}$	.18	.26	.45**
$R_{Y(12)}$	.19	.18	.50**
$R_{Y(123)}$	.71**	.78**	.84**

* $P<0.05$.
** $P<0.01$.

Examination of the effects of litter size and number of fetuses in the uterine horn on fetus weight to ascertain if they acted to any significant extent independently of variation in membrane weight (table 9) has shown that at 25 days of gestation the effect of litter size on fetus weight is direct ($P_{Y1:23}=0.26$, $P<0.05$). The direct effect of number of fetuses in the uterine horn, 0.03, is negligible. By 70 days of gestation, neither of these factors had a significant direct effect on fetus weight. At 105 days of gestation, the direct effect of number of fetuses per horn on fetus weight had become significantly negative ($P_{Y2:13}=-0.27$); the path from litter size to fetus weight had become negligible, 0.12. At all three stages, the three factors upon which fetus weight was considered dependent accounted for a highly significant amount of variation in fetus weight ($R_{Y(123)}=0.71$; 0.78; 0.84 at 25, 70 and 105 days, respectively).

Discussion

The lack of significant differences in 25-day embryo weight associated with differences in feed level is in agreement with the data of Robertson *et al.* (1951) on embryo length. The similar absence of a feed-level effect on fetus weight at 70 and 105 days is not surprising in light of the results of McKenzie (1928) and Vestal (1936).

On the other hand, the difference in feeding regimen has affected the development of the dam significantly as shown by the differences in carcass length, carcass weight, and back-fat thickness. McMeekan (1940) found that in growing animals the final weight of nervous tissue at four months was little affected by severely restricted feed intake; however, the development of bone and muscle was reduced, and the rate of fat deposition was markedly diminished. This would suggest that individual tissues differ in their ability to compete with one another for nutrients. It has been proposed that these differences result from a differential distribution of nutrients which is positively correlated with differences in the metabolic rates of the various tissues (Hammond, 1944). Barcroft (1946) found that the over-all metabolic rates of lamb fetuses were higher than the metabolic rates of many of the maternal tissues throughout much of gestation.

From this it might be expected that in the present experiments a litter should be able to compete with the dam for nutrients on better than equal terms. A moderate restriction in the dam's nutrient intake should then result in retardation of development in maternal tissues only.

The results of Baker *et al.* (1958) and those presented here do not indicate any consistent difference in 25-day embryo weight with breed of dam. Robertson *et al.* (1951), however, noted that both purebred and crossbred embryos from Chester White gilts were significantly longer than those from Poland China gilts. That the 70-day fetuses from Chester White gilts were heavier than those from Poland China gilts may have arisen from a more favorable genetic or maternal environmental reaction of the

Chester White to the Duroc. (This advantage of the Chester White at 70 days was not apparent in the data of Baker *et al.* for Chester White and Poland China gilts, but their gilts averaged approximately 2 months older in age at breeding. The importance of age of dam in this connection is not known). The information at 105 days and term, including data from crossbred litters sired by Chester White, Poland China, and Duroc boars, indicates that at these two stages pigs carried by Chester White gilts are heavier. The data on membrane weight in this study, though not in complete agreement with those of Baker *et al.*, indicate that in Poland China females these tissues may develop more slowly than in Chester White females. Starting rapid growth sooner and reaching their full size earlier, the membranes in Chester White gilts are better able to support fetal growth at an earlier stage. It is further suggested that these differences may be maternal breed characters as the litters carried by these two breeds of gilts are either sired by boars of the same breed or are reciprocal crossbreds.

The changes observed in the relative effects of litter size and number of fetuses in the uterine horn in the present study are consistent with those observed by Baker *et al.* (1958). In both instances, the trend was for litter size to have a positive direct effect on fetus weight at 25 days, over and beyond the association of the two through membrane weight. Also, the number of pigs in the uterine horn appeared to be developing a negative direct effect on fetus weight with the advance of gestation. Membrane weight in both studies showed relatively high direct effects upon fetus weight when the other two factors were held constant. The paths were of the magnitude of 0.60 to 0.80 at the various stages and they were quite comparable in size to the multiple correlations of fetus weight with all three independent variables.

Summary

Differences in maternal environment arising from breed and feeding regimen, as well as individual maternal characteristics, were studied in three trials involving 155 gilts; fetus and placenta weights at 25 days, 70 days, and 105 days of gestation and pig weight at term were considered in these trials. Chester White and Poland China gilts carrying comparable crossbred litters were fed various sequences of two different nutrient levels starting shortly after weaning. Full-feeding was accomplished by self-feeding a low-fiber ration (4–6% fiber); limited-feeding was carried on either by hand-feeding the low-fiber ration at two-thirds the level of self-feeding or by self-feeding a high-fiber ration (11–18% fiber).

Individual differences in maternal growth and fatness had no effect on embryo or fetal development, as shown by path coefficient analysis. Examination of the effect of litter size and number per horn on fetus weight relative to their association with membrane weight indicated that at 25

days litter size has a significant, positive, direct effect on fetus weight; number per horn is unimportant. At 105 days of gestation, some of the effect of number per horn on fetus weight is direct and negative; the direct effect of litter size is negligible. The indirect effects of litter size and number per horn (through membrane weight) on fetus weight were negligible at all stages.

The carcass weight, back-fat thickness, and carcass length of the gilts were reduced by limited-feeding. Pig weights were not affected by the maternal feeding regimen at any of the stages studied. Pig weights near term were greater in litters carried by Chester White gilts than in those carried by Poland China gilts. These greater weights appear to arise in part from the more rapid development of the fetal membranes in the Chester White gilts early in gestation.

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INTERRELATIONSHIPS OF PROTEIN, LYSINE AND ENERGY IN DIETS FOR GROWING SWINE

R. P. ABERNATHY,[1] R. F. SEWELL AND R. L. TARPLEY [2]

University of Georgia,[3] Athens

MITCHELL and Hamilton (1935) demonstrated with rats that an upward adjustment of the protein level, to maintain the proper relation to total energy, is necessary to realize the full benefit of increased energy in the diet. Donaldson (1955) and Sunde (1956), among others, have also demonstrated this with chicks in recent studies. The principle that nutritional economy in animal feeding depends largely upon a correct adjustment between the proportion of protein and total energy supplied is not new. However, recent trends toward the use of higher-energy rations have given new emphasis to this concept.

A similar relationship apparently exists between some of the amino acids and the energy level of the diet. Rosenberg and Culik (1955) presented data indicating that the lysine requirement of the growing rat is a function of the productive energy of the diet. These findings provide a rational basis for a study of lysine supplementation in practical swine diets as related to the energy level of the diet. The experiment reported here was designed to provide information on the interrelationships of protein, lysine and energy in rations for growing pigs.

Experimental

Sixty Duroc and Duroc X Yorkshire weanling pigs averaging about 40 lb. were allotted to 12 comparable groups of five pigs each. These groups were randomly allotted to a 2x2x3 factorial experiment on a completely randomized design in which the main treatment effects were protein, lysine, and energy. The rations were compounded from ground yellow corn, soybean oil meal, peanut oil meal, alfalfa meal, stabilized beef tallow, limestone, defluorinated phosphate, salt and vitamin supplements which contributed the following per lb. of ration: 4 mg. calcium pantothenate, 10 mg. choline chloride, 60 mcg. folic acid, 9 mg. niacin, 2 mg. riboflavin, 10 mcg. vitamin B_{12}, 500 I.U. vitamin A, and 100 I.U. vitamin D_2. All rations were formulated to contain approximately 14 or 18% protein. Soybean oil meal and peanut oil meal were blended in

[1] Present address: Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

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equal parts to supply the protein supplement. L-lysine hydrochloride was added at a level of 0.1% in those diets containing this supplement. Stabilized prime beef tallow was used as a source of fat in varying the energy concentration of the diets. In those diets to which tallow was added, it replaced corn on an equal weight basis and the protein contents were adjusted so that all diets in each protein category were maintained on an isonitrogenous basis. The calculated methionine-cystine content of all diets was adjusted by addition of crystalline dl-methionine to supply a constant level of approximately 3.3% of the dietary protein. Chlortetracycline was added at a level of 10 mg. per lb. to all rations.

The pigs were fed in concrete dry-lot with water and feed supplied *ad libitum*. Individual weight gains and feed consumption for each group were recorded at two-week intervals.

Results and Discussion

The experiment was divided into two 42-day periods for the purpose of determining the effects during the early growth stage and the later finishing stage. An analysis of the combined data was also conducted to determine the over-all treatment effects. Average performance data for all three periods are presented in table 1. Analysis of covariance was used to adjust initial weights to a common weight for the first period and for the combined data since a highly significant correlation for these periods was obtained between initial weight and weight gains. Since no significant correlation between initial weight and weight gain was found for the second 42-day period, no adjustment was made. The plan of the analysis for the three periods is presented in table 2.

A highly significant difference due to treatment was found for the first 42 days which spanned growth approximately from 40 lb. to 110 lb. Pigs receiving the 18% protein rations gained at a significantly faster rate than those receiving 14% protein. Supplementation with 0.1% l-lysine hydrochloride resulted in a highly significant depression in rate of gain during this early growth period. A highly significant linear increase in gains was also obtained as the fat content of the ration was increased with a corresponding increase in caloric density. No significant interaction of protein with fat or lysine supplementation was found for the first period.

During the second 42-day period the pigs increased in weight approximately from 110 lb. to 190 lb. The only differene due to treatment for this period was a highly significant fat linear component. Similar to the pattern for the early growth period, there was a progressive increase in rate of gain with increasing dietary energy level. While no other treatment effect was found to be significant during this period, it is of interest to note that more than half of the remaining variation due to treatment was

TABLE 1. AVERAGE PERFORMANCE DATA FOR FIRST, SECOND, AND COMBINED PERIODS

Ration no.	Factors[a] P %	L %	F %	First 42-day period: Av. daily gain, lb.	Feed per lb. gain, lb.	Daily feed intake, lb.	Second 42-day period: Av. daily gain, lb.	Feed per lb. gain, lb.	Daily feed intake, lb.	Combined period: Av. daily gain, lb.	Feed per lb. gain, lb.	Daily feed intake, lb.
1	14	0.0	0	1.62	2.88	4.67	1.80	3.61	6.50	1.71	3.26	5.59
2	14	0.1	0	1.49	2.77	4.11	1.65	3.75	6.19	1.57	3.28	5.15
3	14	0.0	5	1.71	2.63	4.50	2.07	3.34	6.90	1.89	3.02	5.70
4[b]	14	0.1	5	1.57	2.58	3.47	1.85	3.05	5.62	1.71	2.82	4.55
5	14	0.0	10	1.68	2.42	4.07	2.04	3.02	6.16	1.86	2.75	5.11
6	14	0.1	10	1.73	2.40	4.15	2.00	3.02	6.03	1.87	2.73	5.09
7	18	0.0	0	1.77	2.77	4.89	1.68	3.81	6.41	1.72	3.28	5.65
8	18	0.1	0	1.62	2.86	4.63	1.79	3.65	6.51	1.70	3.27	5.57
9	18	0.0	5	1.73	2.38	4.12	1.78	3.51	6.25	1.76	2.95	5.19
10	18	0.1	5	1.67	2.49	4.14	1.80	3.36	6.06	1.73	2.94	5.09
11	18	0.0	10	1.76	2.39	4.22	1.95	3.18	6.20	1.86	2.81	5.23
12	18	0.1	10	1.73	2.43	4.22	1.92	3.10	5.96	1.83	2.78	5.09

[a] P=Protein; L=Lysine supplementation level; F=Fat supplementation level.
[b] One pig removed from lot 4 because of rectal prolapse after 27 days on trial.

divided about equally between the comparison of protein levels and the protein×lysine component.

When the data for the two periods were combined and analyzed, a highly signifiant linear increase in gains was found as the level of fat was progressively increased. This was the only treatment which exerted a significant effect throughout the experiment. The fat linear component accounted for approximately 99% of the variation due to altered dietary fat level during both periods of growth.

TABLE 2. MEAN SQUARE OF WEIGHT GAINS FOR FIRST, SECOND, AND COMBINED PERIODS

	First 42-day period		Second 42-day period		Combined period	
Variance source	d.f.	Mean square (adjusted)	d.f.	Mean square (unadjusted)	d.f.	Mean square (adjusted)
Total	57[a]		58[a]		57[a]	
Treatment	11	67.14**	11	160.65*	11	320.98*
Protein (P)	1	168.86*	1	166.67	1	0.03
Lysine (L)	1	187.89**	1	68.27	1	492.59
Fat linear (Fl)	1	202.93**	1	1092.03**	1	2249.23**
Fat quadratic (Fq)	1	2.05	1	10.21	1	2.53
P×L	1	1.19	1	187.27	1	154.52
P×Fl	1	31.00	1	34.23	1	124.49
P×Fq	1	6.41	1	95.41	1	150.28
L×Fl	1	98.52	1	0.62	1	81.65
L×Fq	1	8.95	1	29.01	1	69.23
P×L×Fl	1	8.20	1	65.02	1	122.42
P×L×Fq	1	22.62	1	18.41	1	83.30
Error	46	24.56	47	69.26	46	135.69

* Significant.
** Highly significant.
[a] One pig removed because of rectal prolapse. Value calculated by missing plot technique.

The growth-depressing effect due to lysine supplementation obtained during the first 42-day period is not without precedent. Miner *et al.* (1955) reported that levels of supplementary lysine in excess of 0.1% of a corn-cottonseed oil meal ration appeared to depress growth rate in young pigs. Sewell and Abernathy (1955) obtained slight depressions in growth rate of pigs fed rations containing levels of 14 or 17% protein when supplemented with 0.1% l-lysine hydrochloride. Their supplementary source of protein was a blend of equal parts of soybean oil meal and degossypolized cottonseed meal.

In the experiment reported here, lysine supplementation decreased feed consumption markedly on the rations containing no added fat. It appears improbable that this decrease in feed consumption was due to the sensory acceptability of the ration in view of the small quantity of l-lysine employed. Almquist (1947) points out that when an amino acid is not supplied in sufficient amounts, tissue protein synthesis slows down with a resultant accumulation of the other free amino acids in the cir-

culation to be removed by catabolism or excretion. Such an accumulation may result in a depressed appetite which serves to curtail further protein ingestion. Whether a mechanism of this sort, brought about by an imbalance resulting from lysine supplementation, or whether the inhibiting effect of lysine in this instance is a manifestation of a toxicity, is obscure.

It is noteworthy that the difference in feed consumption was reduced when the caloric density of the ration was increased by the addition of tallow. A corresponding decrease in the growth-inhibitory effect of lysine supplementation was also apparent with increasing dietary energy level. Efficiency of feed utilization was progressively increased as the caloric density of the rations was increased.

Live-probe backfat measurements were made on all pigs at the termination of the experiment. Statistical analysis revealed no significant difference due to treatment, although there appeared to be a slight trend toward increased backfat thickness with increasing dietary fat level.

Summary

Sixty pigs were employed in a 2 x 2 x 3 factorial experiment in which the primary treatment effects were protein, lysine, and energy.

During the first 42 days of the experiment significantly faster gains were obtained with pigs fed rations containing 18% protein than with those fed rations containing 14% protein. A highly significant depression in rate of gain resulted when l-lysine hydrochloride was added at a level of 0.1% of the ration. This inhibitory effect appeared to be reduced as the caloric density of the ration was increased. Increasing the caloric density of the ration resulted in a highly significant linear increase in gains with a corresponding decrease in the quantity of feed required to produce a unit of gain.

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THE COMPARATIVE REPRODUCTIVE PERFORMANCE OF NURSED COWS AND MILKED COWS

J. N. WILTBANK [1] AND A. C. COOK [1]

U. S. Department of Agriculture

THE practice of suckling is known to affect the length of the interval from calving to the first post-partum estrus in cows. Clapp (1937) reported that the average length of the interval between calving and first estrus was 71.8 days in nursed cows, compared to 46.4 days for cows milked twice a day and 69.4 days for cows milked four times a day. Suckling also influences the occurrence of estrus in the sow (Burger, 1952; Cole and Hughes, 1946; Heitman and Cole, 1956), rat (Long and Evans, 1922; Seyle and McKeown, 1934), and rabbit (Hammond and Marshall, 1925). The study reported in this paper was undertaken to determine the effects of suckling on ovarian activity, conception rate, and length of the interval from calving to first estrus in a group of Milking Shorthorn cows.

Materials and Methods

The data were collected during the years 1934 to 1957 in a herd of Milking Shorthorn cows at the Agricultural Research Center, Beltsville, Maryland. Prior to 1942, all cows in the herd were milked during each lactation. Since 1941 the general practice has been to milk cows during first lactations and to use them as nurse cows during subsequent lactations. However, some cows were milked one or more lactations after the first. The milked cows were milked twice each day by hand. Calves were allowed to suckle the nursed cows twice a day in the summer and were with them approximately 15 hours each day in the winter.

The feeding and management practices over the years have varied somewhat. Prior to 1949, cows in their first lactation were kept in dry lot and fed a concentrate mixture, hay, and beet pulp. The rest of the milked cows and the nursed cows were on pasture in the summer and were fed hay, grain, and silage in the winter. After 1948, the management of the nursed cows and milked cows was similar. During the winter, all cows were kept in the barn except for approximately 6 hours each day in the exercise lots. They were fed corn silage and hay free choice. During the summer, they were on pasture and were brought to the barn twice each day for milking or nursing.

The quantity and quality of the grain fed the two groups of cows has remained constant over the years. Milked cows received approximately 1 lb. of a concentrate mixture for each 3½ lb. of milk produced (average

[1] Beef Cattle Research Branch, Beltsville, Maryland.

daily production for first 90 days was 26.8 lb. of milk) while nursed cows received 3 to 5 lb. of a concentrate mixture containing a higher proportion of protein.

Checks for estrus were made by the herdsman in the exercise lot or pasture. All cows were hand mated and were usually bred at the first estrus occurring after 50 days post-calving. Service periods (interval from calving to subsequent conception) following abortions were not included in this study.

The study consists of two parts. First, the records kept by the herdsman during the years 1934 to 1955 were studied. These data consist of 405 service periods of 84 cows. Cows used in this part of the study were

TABLE 1. REPRODUCTIVE PERFORMANCE OF NURSED COWS AND MILKED COWS, 1934 TO 1955

Items	Nursed cows	Milked cows	Difference
Number of service periods	266	139	
Service period, av. no. days	152(85.4)	94(57.0)	58**
Calving to first estrus, av. no. days	104(48.4)	74(35.1)	30**
First service to conception, av. no. days	47(71.9)	20(42.9)	27**
Av. no. services per conception	1.84(1.28)	1.54(1.07)	0.30**

Figures in parentheses are standard deviations.
** Significant at the 1% level.

nursed at least one service period and milked for at least one service period. Because cows were not nursed prior to 1942, all cows which were culled from the herd before this date were thus excluded from this study. Only complete service periods were used.

The second part of the study includes the records from late 1955 through 1957. Cows were checked more systematically for estrus, and regular rectal examinations of the genital organs were made during this period of time. The data consist of 69 service periods of 57 cows. Cows having two service periods included may have been either milked or nursed during both. Because of a change in the breeding program in 1956, many of the cows were not bred as soon as possible after calving during this period. Therefore, only the interval between calving and first estrus was studied. Rectal examinations were carried out at weekly intervals beginning 15 to 21 days after calving and ending after the uterus was involuted and the cow showed estrus.

Results

Records from 1934 to 1955. The average length of the service period was 152 days for the nursed cows and 94 days for the milked cows ($P<0.01$) (table 1). A breakdown of the service period revealed that the average interval from calving to first observed estrus was 30 days longer

in the nursed cows ($P<0.01$) and the average interval from first service to conception was 27 days longer ($P<0.01$). These data were analyzed by using the method for disproportionate subclass numbers with interaction negligible outlined by Snedecor (1950). The correlation between the interval from calving to first estrus and the interval from first service to conception was 0.06 for the milked cows and —.05 for the nursed cows. These small correlations fail to suggest an effect of the time interval from calving to first estrus on conception rate.

The lowered fertility in the nursed cows, as evidenced by the longer interval between first service and conception, was the result of more services being required on the average for each conception and a longer interval between services (return interval). The nursed cows required

TABLE 2. THE NUMBER AND THE PERCENT OF COWS PREGNANT AFTER VARIOUS NUMBERS OF SERVICES

Type of cow	No. service periods	After one service	After two services	After three services
Milked	139	99(71%)	119(86%)	130(94%)
Nursed	266	152(57%)	208(78%)	243(91%)

significantly more services per conception (1.84 compared to 1.54 for milked cows, ($P<0.01$) (table 1). This greater average number of services per conception was largely the result of an apparent difference in fertility at the first service. Ninety-nine of the 139 milked cows (71%) became pregnant at first service compared to 152 of the 266 nursed cows (57%). This difference when tested by the square approached significance ($P\cong0.07$) (table 2). After the second service 86% of the milked cows were pregnant and 78% of the nursed cows. The proportion of cows pregnant after the third service was 94% for the milked cows and 91% for the nursed cows.

The average length of all return intervals (interval between services) was 58 days in the nursed cows and 37 days in the milked cows ($P<0.05$) (table 3). However, suckling was significantly associated with a lengthened return interval only following the first service. This return interval averaged 68 days in the nursed cows and 34 days in the milked cows ($P<0.01$). The average length of the return interval following all services except the first was 46 days in the nursed cows and 40 days in the milked cows ($P>0.05$). The return intervals following first service vs. those following later services were differentially affected by the method of milk removal ($P<0.01$). Return intervals in the nursed cows exceeded 110 days in 20% of the cases following the first service compared to 6% of the cases following later services while the corresponding figures for the milked cows were 2 and 3% ($P<0.01$) (table 4). No significant association

TABLE 3. AVERAGE LENGTH OF THE INTERVAL BETWEEN SERVICES (RETURN INTERVALS)

Return intervals	Nursed cows		Milked cows		Difference
	No. of intervals	Average no. days	No. of intervals	Average no. days	Average no. days
Following all services	210	58	76	37	21*
Following 1st service	114	68	40	34	34**
Following all services except the first	96	46	36	40	6
Difference		22**		6	

* Significant at the 5% level.
** Significant at the 1% level.

between interval from calving to first estrus and length of the return interval between first and second service was found (correlation —0.06 in nursed cows and —0.28 in milked cows).

There was a significant difference between cows in the length of the interval between calving and first estrus ($P<0.01$) and the length of the service period ($P<0.01$). The tendency for a cow to repeat her reproductive performance was determined from repeatability figures (intraclass correlations). These correlations were calculated for the service periods of the nursed cows and the milked cows separately, and for all service periods. Repeatability for the service period was 0.00, 0.14, and 0.11 for the service periods in which a cow was suckled, milked, and for all service periods, respectively. Repeatability for the interval from calving to first estrus was 0.12, 0.05, and 0.06 for the nursed, milked, and all service periods, respectively. Comparable figures for the interval from first service

TABLE 4. DISTRIBUTION OF THE RETURN INTERVALS

Items	Total no.	Under 17 days	17–24 days	25–50 days	51–70 days	71–110 days	Over 110 days
Milked cows		%	%	%	%	%	%
Intervals following 1st service	40	10	40	38	10	0	2
Intervals following all services except the first	36	6	33	42	14	3	3
Nursed cows							
Intervals following 1st service	114	2	25	23	15	16	20
Intervals following all services except the first	96	7	38	23	12	14	6

to conception were 0.00, 0.03, and 0.00 for the milked, suckled, and all service periods, respectively. Thus the repeatability of these items of reproductive performance was small and statistically insignificant.

The effect of age on reproductive performance was studied by arraying the service periods according to the lactation in which they occurred (first, second, etc.). Cows were not nursed during the first lactation. The nursed cows had a longer interval between calving and first estrus than the milked cows in the second through the seventh lactation (table 5). There was a tendency for this interval to shorten as the cow grew older in both the milked cows ($P<0.01$) and the nursed cows ($P<0.05$). The records of 19 nursed cows which had records in the second through the sixth

TABLE 5. THE REPRODUCTIVE PERFORMANCE OF COWS IN DIFFERENT LACTATIONS

Lactation no.	Number of service periods		Calving to 1st estrus (av. no. days)		1st service to conception (av. no. days)		Average no. services per conception	
	Milked	Nursed	Milked[b]	Nursed[a]	Milked	Nursed	Milked	Nursed
1	82	0	87	...	24	..	1.66	....
2	19	58	56	119	15	43	1.52	1.62
3	13	50	55	116	24	45	1.38	1.88
4	8	45	53	111	8	48	1.12	1.91
5	7	32	58	93	7	46	1.14	1.97
6	4	29	72	95	0	33	1.00	1.66
7th and over	6	52	45	83	15	63	1.67	2.17

[a] Differences between lactations significant at the 5% level.
[b] Differences between lactations significant at the 1% level.

lactation and of 12 milked cows having records in the first through the third lactation were examined. These records showed the same trend as shown in table 5. Thus, factors other than culling were apparently responsible for the shortening of the interval from calving to first estrus with increasing age. The interval from first service to conception was longer for nursed cows than for milked cows in each lactation where a comparison could be made and the nursed cows required more services per conception in each lactation from the second through the seventh. Although the overall difference between nursed and milked cows for the interval from first service to conception and for number of services required for conception was significant, the difference between nursed and milked cows in the second and third lactation for these two observations was not significant.

The season in which a cow calved did not influence reproductive performance significantly. The average length of the interval between calving and first estrus for cows calving in the winter, spring, summer, and fall was 98, 90, 88, and 100 days, respectively ($P>0.05$). The average length of the interval between first service and conception was 38, 51, 32, and 32 days for cows calving in the winter, spring, sum-

mer, and fall, respectively ($P>0.05$). The year in which a cow calved did not significantly influence the length of the interval from calving to first estrus or the length of the interval from first service to conception. The level of milk production was not related significantly to reproductive efficiency. The correlation between pounds of milk produced during the first 90 days of lactation and the interval from calving to first estrus was —0.06 for all lactations of the milked cows. For the first lactation the correlation was 0.20 and approached significance at the 5 percent level.

TABLE 6. REPRODUCTIVE PERFORMANCE OF NURSED COWS AND MILKED COWS—1955 TO 1957

Item	Nursed cows	Milked cows	Difference
All cows			
Number of service periods	34	35	
Calving to 1st estrus, av. no. days	84(41.2) [a]	54(25.2)	30**
Calving to 1st corpus luteum, av. no. days	53(18.3)	36(15.6)	17**
1st corpus luteum to 1st estrus, av. no. days	30(37.0)	17(22.2)	13
Average no. ovulations before 1st estrus	1.56(1.83)	0.91(1.07)	0.65
Calving to involution, av. no. days	56(15.6)	44(11.7)	12**
Cows having quiet ovulation			
Number	20	20	
Calving to 1st corpus luteum, av. no. days	56	35	21**
1st corpus luteum to 1st estrus, av. no. days	51	30	21*

[a] Figures in parentheses are standard deviations.
* Significant at 5% level.
** Significant at 1% level.

Records From 1955 to 1957. The average length of the interval between calving and first estrus was 84 days for the nursed cows and 54 days for the milked cows (table 6). This difference of 30 days was significant ($P<0.01$) and approximated the difference in the earlier records. However, the average length of this interval was about 20 days shorter in each group of cows than it was in the earlier records (104 vs. 84 days for nursed cows and 74 vs. 54 days for milked cows).

When the difference in the length of the interval from calving to first estrus of 30 days was broken into its component parts it showed that the interval from calving to first corpus luteum averaged 17 days longer in the nursed cows ($P<0.01$) and that the interval from first corpus luteum to first estrus averaged 13 days longer ($P>0.05$). The nursed cows had 0.65 more ovulation without an observed estrus ($P>0.05$) before the first post-partum estrus (quiet ovulation). Twenty of 34 nursed cows and 20 of 35 milked cows had one or more quiet ovulations. The length of the interval between calving and detection of the first corpus luteum was approximately the same in cows having quiet ovulations as it was in cows which had no quiet ovulation in both the milked and the nursed

cows, (56 and 53 days for the nursed cows and 35 and 36 days in the milked cows) (table 6). The average time from calving to involution of the uterus was 56 days in the nursed cows and 44 days in the milked cows ($P<0.01$) (table 6).

Discussion

The reason for the difference in reproductive efficiency between the nursed and the milked cows is open to speculation. The majority of the service periods studied for the milked cows were first lactations while those studied for the nursed cows were second or later. However, it appears that age did not cause the difference between the milked cows and the nursed cows in the reproductive phenomena studied in the earlier records. In fact, it appears that the difference between the two groups tended to diminish with increasing age in some instances. Other factors which did not influence reproduction significantly in the early records were the season of calving, the year of calving and milk productivity. The difference in amount of concentrates received by the two groups of cows might influence reproductive efficiency to some degrees; however, it was impossible to separate the effects of feed and method of milk removal.

When the cows were checked more closely for estrus in the second part of the study, the apparent interval between calving and first estrus was approximately 20 days less in both groups of cows than it was in the earlier years when heat checks were less systematic. Whether this apparent shortening was due to unobserved estrus periods prior to 1955 or to a real change, it was approximately the same in the milked and nursed groups. The effect of age, year of calving, season of calving, and milk production on reproductive performance in the second part of the study was not studied because of the limited number of observations. If these factors did not influence reproduction significantly in the second part of the study, then suckling would be the best explanation for the delay in ovarian activity, as measured by the longer interval from calving to first corpus luteum in the nursed cows. Although not statistically significant, there was a tendeny for suckling to be associated with a greater incidence of quiet ovulations before the appearance of the first post-partum estrus. Observers working in other species have pointed out that suckling decreases ovarian activity and that injection of gonadotrophic hormone in suckled animals results in increased ovarian activity. Warnick, *et al.* (1950) found that the average follicular volume was smaller in suckled sows than in non-suckled sows. Cole and Hughes (1946) and Heitman and Cole (1956) reported that pregnant mare serum injected into lacting sows after the 39th day of lactation caused estrus quite consistently and most sows which were bred conceived readily. Whitten (1955) showed

that injection of gonadotrophic hormone into lactating mice caused an increase in the ovarian weight to approximately that of the non-lactating mice. Whitten (1955) also showed that an injection of either gonadotrophic hormone or estrogen into lactating mice caused implantation to occur.

The difference in reproductive performance between the two groups of cows might be either the result of a decreased supply of gonadotrophic hormone or an insensitivity of the ovaries to ordinary levels of gonadotrophin. Some evidence suggests that the level of gonadotrophic hormone is affected in lactating animals rather than the sensitivity of the ovary. Desclin and Grégorie (1937) grafted one ovary from an immature rat into the kidneys of a spayed lactating rat and the other ovary into a spayed non-lactating rat. The ovarian implants remained small and relatively non-active in the spayed lactating animals while the ovarian implants in the spayed non-lactating rats grew tremendously and were filled with corpora lutea. Also, the typical changes caused by castration were not present in the pituitary of the spayed lactating rats but were present in the spayed non-lactating rats. While there is a species difference, the evidence available at least suggests that a decreased supply of gonadotrophic hormone in the nursed cows could be responsible for their lower reproductive performance.

The increase in the number of quiet ovulations in the nursed cows (1.56 vs. 0.91 for the milked cows) was not statistically significant. However, the average interval between calving estrus was 13 days longer. This increase in the number of quiet ovulations might be due to levels of estrogen sufficient to cause ovulation but insufficient to bring about estrus, or to a condition of relative insensitivity to estrogen stimulation.

The longer interval from first service to conception in the nursed cows (47 days as compared to 20 days in the milked cows) was due in part to the fact that a smaller proportion of the nursed cows settled at first service (57% compared to 71%), thus showing that fertilization failure or embryonic death rate or both were higher in the nursed cows. The 21-day longer return interval in the nursed cows was important in determining the relative reproductive efficiency of the nursed and milked cows. This long return interval could be the result of persisting corpora lutea, of embryonic death taking place at a later date, of quiet ovulations or of ovaries returning to an inactive state.

The longer time (12 days) required by the nursed cows for involution of the uterus could be due to the fact that all of the service periods of the nursed cows occurred in the second or later lactations while the majority of service periods for the milked cows occurred in the first lactation. Buch, *et al.* (1955) pointed out that the average time from calving to involution of the uterus was 50 days in pluriparous cows but only 42 days in primiparous cows. However, if suckling does affect hormone levels the time required for involution might also be affected.

Summary

In a herd of Milking Shorthorns the average length of service period was 152 days in nursed cows and 94 days in cows milked twice a day. The interval from calving to first estrus was approximately 30 days longer in the nursed cows and the interval from first service to conception was 27 days longer. The average length of interval from calving to first corpus luteum was 53 days in the nursed cows and 36 days in the milked cows. Nursed cows averaged 0.65 more quiet ovulations before the first postpartum estrus and had a longer interval from first corpus luteum to first estrus (30 days compared to 17 days in the milked). The number of services required per conception was 1.84 for the nursed cows and 1.54 for the milked cows. A larger proportion of the milked cows settled at the first service (71% compared to 57% for the nursed cows). The interval between services averaged 58 days in the nursed cows and 36 days in the milked cows. The results indicate that the reproductive efficiency of cows milked twice daily is considerably better than that of cows nursing calves.

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SOME FACTORS AFFECTING CARCASS GRADE IN STEERS[1]

W. T. MAGEE, R. H. NELSON, G. A. BRANAMAN, L. J. BRATZLER AND A. M. PEARSON

Michigan State University,[2] [3] East Lansing

IN recent years, there has been increased interest in enlarging the area of the loin eye in steers. However, there is little information published on what effect such an increase would have on carcass grade.

Estimates of heritability for carcass grade and area of the loin eye have been reported. Estimates for heritability of carcass grade have been 0.16 by Shelby *et al.* (1955), 0.84 by Knapp and Nordskog (1946) and 0.33 by Knapp and Clark (1950). Estimates of heritability for loin eye area obtained by the above authors have been 0.72, 0.69 and 0.68, respectively. These estimates were obtained from data on Montana cattle and, therefore, may not be reliable estimates of the traits for other cattle, such as those in the North Central region.

Primarily this study had two purposes: first, to determine the relationship between carcass grade and some of the measurable traits of a steer; and second, to estimate the heritability of carcass grade and loin eye area of cattle raised in Michigan.

Procedure

Source of Data. For the relationship phase of the study, data were used from 62 steers produced at the Lake City Branch Experiment Station. The cattle raised at Lake City are used in Michigan State University's contributing project to the NC-1 Beef Cattle Breeding Project. As related to the steers used in this study, the project operates in the following way. Each year, four performance-tested bulls (one Angus and three Herefords) are selected and mated to 15 to 18 cows of their respective breeds. The following fall, representative steers from each sire are taken to the feed lots at East Lansing and individually fed to the estimated average grade of low choice. The first year, 1953–54, the steers were group fed, and no Angus steers were available. The first two years the steers were slaughtered at a packing plant; whereas, the last two years, they were slaughtered at the University meat laboratory. Carcasses were graded in the ribbed carcass to the nearest one-third of a grade by a U.S.D.A. grader. Most of the carcasses were in the choice grade but a few were either prime or good. The performance of the steers by sire groups is shown in table 1.

[1] Journal Article No. 2178 from the Michigan Agricultural Experiment Station.

[2] Department of Animal Husbandry.

[3] This study was conducted in cooperation with the North Central Regional Project NC–1, The Improvement of Beef Cattle Through Breeding Methods.

TABLE 1. MEAN PERFORMANCE, BY SIRE GROUPS, OF STEERS FROM LAKE CITY

Years	Sire no.[a]	No. of steers	Final age	Final wgt.	Days on feed	Gain	Area of loin eye	Carcass grade[b]	Adj. loin eye[c]	Adj. carcass grade[d]
	1	6	437	779	180	357	8.56	10.3	8.67	10.5
1953–54	2	3	428	843	180	365	9.35	11.0	9.13	10.7
	3	6	429	798	180	356	9.37	10.5	9.39	10.5
	5	4	474	882	264	482	11.15	9.5	11.17	9.5
1954–55	6	4	473	876	264	485	9.21	11.0	9.25	11.1
	7	4	455	898	264	548	10.39	10.8	10.33	10.7
	8A	4	505	899	264	459	10.84	11.5	10.77	11.4
	9	4	449	803	210	394	8.27	9.8	8.60	10.2
1955–56	10	4	449	916	210	479	8.70	10.2	8.46	9.9
	11	4	430	869	210	478	9.21	10.0	9.20	10.0
	12A	4	442	884	210	442	9.96	12.2	9.84	12.1
	13	3	420	873	215	464	7.62	9.7	7.76	9.9
1956–57	14	4	410	867	215	468	8.27	10.0	8.44	10.2
	15	4	429	974	215	534	8.91	10.5	8.53	9.9
	16A	4	434	886	215	456	9.07	11.2	9.14	11.3

[a] A denotes an Angus sire. The Angus cows at Lake City are a more selected group than the Hereford cows.
[b] U. S. carcass grades were converted to numerical values as follows: Prime+=15, Prime (Av.)=14, etc.
[c] Adjusted for the regression of loin eye on final weight, b=.0051, to approximately the average final weight of the Hereford steers each year.
[d] Adjusted as in 3 with b=.0074.

Data from an experiment reported by Deans *et al.* (1956) were also used in the relationship phase of the study. In this experiment there were 42 purchased Western steers divided equally into three experimental groups. The exact age of individual steers was unknown. The steers had an average final weight of 1157 lb. and were fed 98 days on test.

For the heritability phase of the study, only data from the Hereford steers raised at Lake City were used. The procedure places the estimates of the genetic effects on a within breed basis, the form in which they are generally expressed.

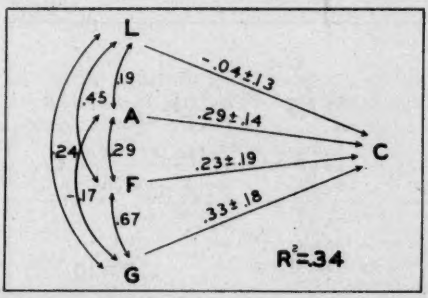

Figure 1. The relationship between carcass grade (C) and area of loin eye (L), age (A), final weight (F) and gain (G) for Lake City steers.

Method of Analysis. All of the data were analyzed on a within breed-year group or on a within treatment group basis. In the first phase, standard partial regression coefficients were calculated following the procedure outlined by Snedecor (1946). Shelby *et al.* (1955) give a complete discussion of the use of paternal half-sib correlations to calculate heritability estimates.

Results

The relationship between carcass grade and age, final weight, gain and area of loin eye is shown for the Lake City steers in figure 1 as a path coefficient diagram, after Wright (1934). The straight arrows represent the path coefficients and are the same as standard partial regression coefficients. The curved arrows represent correlation coefficients.

A measure of the direct effect of each of the independent variables on carcass grade is the square of each of the path coefficients. (Wright, 1934). Thus, gain had the largest direct effect but accounted for only .11 of the variation in carcass grade. All of the independent variables combined accounted for 0.34 of the variation.

The —.04 path coefficient between area of loin eye and carcass grade indicates that on carcasses from steers of the same weight, age and gain, the

area of the loin eye had no effect on carcass grade. However, the correlation between area of loin eye and carcass grade was 0.20 since loin eye was positively associated with age, weight and gain, and each of these had a positive effect on carcass grade. The correlations for the other traits with carcass grade were as follows: age, 0.29; weight, 0.52; and gain, 0.43.

The relationship between carcass grade and the other variables, excluding age, for the steers reported by Deans *et al.* (1956) is shown in figure 2. Here again, with steers of uniform weight and gain, there was no

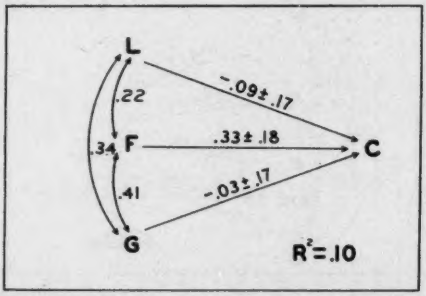

Figure 2. The relationship between carcass grade (C) and area of loin eye (L), final weight (F), and gain (G) for Deans' steers.

relationship between area of the loin eye and carcass grade. Also, the correlation between carcass grade and area of the loin eye, not adjusted for the other variables, was —.03. For these cattle which were fed a shorter time, final weight had a larger effect than gain on the carcass grade of the steers. The direct effect of final weight was 0.11. Area of loin eye, gain and final weight combined only accounted for 0.10 of the variation in carcass grade, since area of loin eye and gain each had a negative direct effect on carcass grade but were positively correlated with final weight.

Heritability estimates in this study were obtained from paternal half-sib comparisons. The more half-sibs resemble each other compared to unrelated animals for a particular trait, the higher heritability is for that trait. The numerical estimate of the size of the heratibility is four times the intraclass correlation. An estimate of the intraclass correlation for carcass grade in the steers from Lake City can be obtained from table 2. Since the mean square for sires was less than the mean square for steers within sires, the intraclass correlation would be negative. This indicates that genic differences among the sires were small compared to other sources of variation and sampling errors have caused the estimate of the variance component for sires, V(S), to be negative. This indicates

that heritability of carcass grade in these cattle was near 0. This estimate of heritability, like other estimates of heritability based on half-sib relationships, is subject to large sample errors since the sampling errors in the estimate of the intraclass correlation are multiplied by 4 in making the estimate of heritability. The data analyzed in table 2 have been adjusted for final weight. The data were not adjusted for both age and weight because the correlation between carcass grade and weight was 0.52 and including age as an additional independent variable only increases the multiple correlation of grade with both weight and age to 0.58. The analysis of the data unadjusted for final weight also indicates that the heritability of carcass grade was small.

TABLE 2. ANALYSIS OF VARIANCE OF CARCASS GRADE AND AREA OF LOIN EYE, EACH ADJUSTED FOR FINAL WEIGHT

Source of variance	d.f.	Mean square		Expected mean square
		Grade	Loin eye	
Years	3	1.03	8.19**	
Sires/Years	8	.99	1.40**	V(E)+4.17 V(S)
Within sires	38	1.04	.38	V(E)

For Loin Eye: V(E)=.38, V(S)=.24, r_I=.39

** Significant at 1% level.

The estimate of heritability of area of loin eye was obtained from the analysis of variance (table 2). The intraclass correlation multiplied by 4 gives an estimate of 1.56. This is above the biological limit of one for the heritability but indicates that heritability of area of loin eye was near one. Again, these data were adjusted only for weight.

Even with this high estimate of heritability for area of loin eye, the differences between the three sire groups each year were statistically significant in only one year. This points up the fact that where only a few sires are used each year, a person should not expect to find a significant difference between the means of the sire groups in only one year. This condition is imposed because the only thing that makes the mean square between sires larger than the mean square within sires is k_o times V(S), the variance component for sires, where k_o is the average number of offspring per sire as calculated by Snedecor (1946). In a random breeding population, V(S) contains only one-fourth of the genic variance and the genic variance is often less than half of the variance in a population. Thus, V(S) is much less than the V(E), the within sire mean square. In a population where heritability is 0.5, the ratio of V(E) to V(S) is 7 to 1. If three bulls are used from this population each year, the number of offspring per bull will have to be 16 or more to obtain a significant difference between sires at least half of the time.

Discussion. Age, final weight, and gain appeared to have a positive direct effect on the carcass grades of yearling steers fed 180 days or longer. However, for predicting the carcass grade of a steer from these variables, little was gained by considering more than final weight. The correlation between weight and carcass grade was 0.52 while the multiple correlation of carcass grade with age, weight and gain was only 0.58. The regression of carcass grade on weight was 0.0074. Thus, within these cattle, which were managed alike throughout their life, the faster gaining, heavier steers tended to produce carcasses which graded higher because of increased quality grade factors.

Apparently sampling errors have made the estimate of heritability of carcass grade below its true value and the estimate of heritability of area of loin eye above its true value. Nevertheless, the estimates that heritability was low for carcass grade but high for area of loin eye agree with the estimates made by Shelby (1955). Knapp and Nordskog (1946) and Knapp and Clark (1950) found heritability of both traits to be high.

The low estimate of heritability for carcass grade in this study indicates that the differences between the sires used were not large enough to affect carcass grade. Thus, it appears that there must be large differences among the sires before any method of selection will be effective in increasing the carcass grade of the offspring produced.

The high estimate of heritability for area of loin eye indicates that a breeder can make some increase in the area of the loin eye of his cattle by selecting for use in his breeding herd the parents and sibs of the steers with large loin eyes. However, the relationship between carcass grade and area of loin eye indicates that this increase in loin eye would not at present be accompanied by an increase in carcass grade.

Summary

Data from 62 yearling Hereford and Angus steers which were fed 180 days or longer and managed alike throughout their life were used in the first part of the study. In these data, gain had a larger direct effect than age, final weight, or area of loin eye on the carcass grade of the steers, accounting for 0.11 of variation in carcass grade. All the factors taken together accounted for 0.34 of the variation in carcass grade. The direct effect of area of loin eye on carcass grade was small, 0.0016, and negative. However, the correlation between area of loin eye and carcass grade was positive, 0.20, because area of loin eye was positively correlated with age, final weight, and gain, and each of these was positively associated with carcass grade. Carcass grade was more highly correlated with final weight (correlation coefficient equaled 0.52) than with any of the other variables.

Data from 42 older and larger Hereford steers fed 98 days were also analyzed. In these data, only final weight had a significant direct effect

on carcass grade, accounting for 0.11 of the variation. Age of the individual steers was not known.

Heritability estimates were obtained for carcass grade and area of loin eye using data from the Hereford steers mentioned in the first part. The estimates indicate that heritability is low for carcass grade and high for area of the loin eye.

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THE EFFECT OF CHLORTETRACYCLINE FEEDING ON CELLULOSE DIGESTION IN THE BOVINE RUMEN,[1] [2]

M. R. LAMBERT [3] AND N. L. JACOBSON

Iowa Agricultural Experiment Station, Ames

THE growth-stimulating effect of chlortetracycline feeding to the young bovine has been demonstrated by many investigators, but the effects upon the older animal, particularly as regards cellulose digestion, are less clear. Murley and associates (1952) and Lassiter *et al.* (1955) found that this antibiotic had no major effect upon digestion of feed nutrients by dairy calves. Bell *et al.* (1951) reported that chlortetracycline (200 mg. daily) caused a decrease in crude fiber digestion by steers. Horn and associates (1955) found 100 mg. of chlortetracycline per steer daily depressed roughage digestion by microorganisms but 32 mg. of the antibiotic did not. Evans and associates (1957) reported that feeding chlortetracycline to sheep decreased the apparent crude fiber digestibility only if fed at a level greater than 1 mg. per lb. of feed. Experiments by Hungate *et al.* (1955) indicated that chlortetracycline feeding altered the composition of the rumen microbial population of steers but did not alter appreciably the potential for microbial activity. Bartley *et al.* (1953) reported that chlortetracycline feeding at levels of 320 mg. per 1000 lb. body weight daily had no adverse effect on milk production, but higher levels apparently reduced feed consumption. Jacobson and associates (1954) fed 240 mg. of chlortetracycline daily to one heifer in each of 17 pairs for a minimum of 15 months prior to parturition and during the first lactation and found no appreciable differences between groups in milk or fat production. Perry *et al.* (1954) reported that yearling steers fed 75 mg. chlortetracycline daily gained weight faster than control animals. Kesler and Knodt (1952), however, determined by use of the artificial rumen that microorganisms from oxytetracycline-fed calves digested less cellulose than those from unsupplemented calves. Similarly, Lodge *et al.* (1956), Lambert and Jacobson (1956), and Hanold *et al.* (1957) found that *in vitro* cellulose digestion by microorganisms from animals fed chlortetracycline was less than that by microorganisms from animals receiving no antibiotic. Since cellulose has an important role in the nutrition of ruminants, it seemed desirable to clarify further the *in vivo* effect of chlortetracycline upon cellulose digestion in the rumen.

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[2] Supported in part by funds provided by American Cyanamid Co., Pearl River, N. Y.

[3] Present address: Western Condensing Co., Appleton, Wisconsin.

Experimental Procedure

Three steers each fitted with a rumen fistula, were employed in this study. At the beginning of the first experiment their ages were: 3903, 29 mo.; 4177, 18 mo.; and 4191, 18 mo. The control diet consisted of good quality alfalfa hay fed *ad libitum* and 2 lb. of concentrate (37% corn, 30% oats, 20% wheat bran, 10% soybean oil meal, 2% steamed bone meal and 1% salt) per animal per day. The experimental diet differed only in that it contained Aurofac 2A mixed with the grain to provide approximately 13 mg. chlortetracycline per 100 lb. body weight daily. Nylon bags, each containing 2.850 gm. (moisture-free basis) of cellulose,[4] were introduced into the rumens through the fistulas, and cellulose digestion (as estimated by disappearance from the bags) was determined at various intervals.

In the first experiment two treatments (control vs. chlortetracycline) were used alternately for 3-week periods with a total of four control and three antibiotic periods. Cellulose digestion after 48 hours was determined at weekly intervals. Four nylon bags were used concurrently for each animal: three contained known quantities of cellulose, and the fourth served as a control.

In the second experiment the same three animals, 3903, 4191, and 4177, served as controls for 6, 10, and 14 weeks, respectively, preceding a 12-week period of antibiotic feeding. The final control period was 10, 6, and 2 weeks for the respective animals. At 2-week intervals four bags were placed in the rumen of each animal, and one was removed for analysis after each of the following periods: 24, 48, 72, and 96 hours.

Each bag suspended in the rumen was weighted with a 10 gm. metal nut and secured to the fistula plug with nylon string. When each bag was taken from the rumen it was washed carefully in cold water to remove any feed particles which might have adhered.

To determine the cellulose content, each bag was placed in a 100 ml. beaker. Forty-eight ml. of glacial acetic acid and 6.0 ml. of concentrated nitric acid were added. The beakers were placed in a boiling water bath for 20 minutes; then the contents, while hot, were filtered into Gooch crucibles. The sediment was washed from the beakers with 95 percent ethanol. The samples (in the crucibles) were dried at 100° C., cooled in a desiccator, weighed, and then ashed in a muffle furnace at a temperature of 600° C. The crucibles again were cooled and weighed to determine the amount of cellulose. The difference between the initial amount (2.850 gm.) and that after *in vivo* digestion was employed as a measure of cellulose digestion. The control (nylon bag containing no cellulose) was completely solublized by the acid to give a value of essentially zero in all instances, indicating no appreciable passage of cellulose from the rumen contents into the nylon bags.

[4] Solka-Floc produced by Brown Co., Berlin, N. H.

Results and Discussion

The effect of chlortetracycline on *in vivo* cellulose digestion by rumen microorganisms in experiment I is indicated in table 1. Two animals, 3903 and 4191, digested cellulose slightly more efficiently when chlortetracycline was not fed; however, 4177 digested cellulose most efficiently

TABLE 1. EFFECT OF CHLORTETRACYCLINE FEEDING ON CELLULOSE DIGESTION (EXPERIMENT I)[a]

Weeks	Treatment[b]	Steer no.			
		3903	4177	4191	Mean
1	C	67.0	31.5	46.9	48.5
2	C	38.6	37.6	47.2	41.1
3	C	59.7	43.5	37.9	47.0
4	A	43.2	45.3	49.1	45.9
5	A	34.9	58.2	44.4	45.8
6	A	53.3	43.7	47.2	48.1
7	C	50.0	38.8	60.2	49.7
8	C	52.2	28.5	39.2	40.0
9	C	66.7	54.9	67.4	63.0
10	A	64.5	71.2	58.6	64.8
11	A	49.8	42.0	47.5	46.4
12	A	60.9	54.3	45.5	53.6
13	C	59.6	55.7	45.4	53.6
14	C	56.8	52.7	54.0	54.5
15	C	50.4	46.6	57.3	51.4
16	A	51.0	65.6	47.2	54.6
17	A	41.5	69.8	42.8	51.4
18	A	45.4	38.8	59.3	47.8
19	C	37.1	58.6	60.8	52.2
20	C	39.5	66.2	54.5	53.4
21	C	46.3	52.7	41.5	46.8

[a] Each value is the average percent for three samples after 24 hours digestion.
[b] C=control; A=chlortetracycline.

during antibiotic feeding. After 48 hours the mean values for all observations were 50.1% cellulose digestion when no antibiotic was administered and 50.9% when the antibiotic was fed to the same animals. Insofar as possible uniform conditions were maintained throughout the experiment. There was a gradual increase in cellulose digestion (mean values by periods for all animals) to the 12th week, and a gradual decline during the following 9 weeks. The cause of these trends was not clear. Analysis of variance (Snedecor, 1956) showed the difference in cellulose digestion between the two treatments not to be significant at $P=0.05$.

In experiment II chlortetracycline feeding to the animals was initiated at varied time intervals from the start of the experiment, but each animal received the antibiotic for 12 successive weeks. This procedure was

TABLE 2. EFFECT OF CHLORTETRACYCLINE ON CELLULOSE DIGESTION (EXPERIMENT II)

		Treatment[a]								
		Control				Chlortetracycline				
Steer no.	Weeks	24 hr.[b]	48 hr.	72 hr.	96 hr.	Weeks	24 hr.	48 hr.	72 hr.	96 hr.
		(Percent cellulose digested)					(Percent cellulose digested)			
3903	2	18.4	35.9	65.2	c					
	4	27.7	46.3	55.7	62.9	8	19.6	64.1	c	c
	6	27.3	39.9	45.6	c	10	28.4	34.7	35.7	74.7
						12	21.3	50.8	61.4	65.0
	20	27.0	39.7	59.6	74.5	14	41.0	48.4	62.0	81.0
	22	27.4	41.0	61.6	68.6	16	33.6	53.6	61.0	70.5
	24	38.7	52.4	85.1	c	18	29.7	35.9	55.0	62.8
	26	29.8	63.5	73.8	79.5					
	28	35.5	43.3	58.9	70.8					
Mean		29.0	45.2	63.2	71.3		28.9	47.9	55.0	70.8
4177	2	25.2	52.4	92.6	c					
	4	28.4	52.6	65.2	c					
	6	25.2	44.9	46.7	62.0	12	24.4	41.5	56.1	c
	8	29.5	80.2	89.9	c	14	28.4	42.0	52.7	64.4
	10	28.9	43.3	61.9	63.3	16	32.8	42.5	c	c
						18	15.7	18.8	38.3	c
	24	28.4	36.8	48.4	57.8	20	32.9	49.2	55.5	c
	26	25.7	44.8	66.3	c	22	27.3	31.5	47.3	66.3
	28	30.5	40.6	49.1	64.9					
Mean		27.7	49.4	65.0	62.0		26.9	37.6	50.0	65.4
4191	2	34.7	51.7	66.3	c					
	4	27.3	41.5	63.1	c					
	6	29.1	d	48.4	56.8	16	35.8	68.4	90.5	c
	8	31.3	51.1	55.2	65.9	18	25.9	58.4	c	c
	10	44.2	66.3	88.4	92.6	20	19.7	c	c	c
	12	30.5	35.7	50.5	58.9	22	22.0	29.4	34.7	70.5
	14	35.9	43.7	58.9	73.3	24	15.9	33.0	47.4	59.6
						26	51.5	64.2	70.5	75.7
	28	35.5	47.3	56.0	69.4					
Mean		33.6	48.2	60.8	69.5		28.5	50.7	60.8	68.6
Mean all animals		30.1	47.6	63.0	67.6		28.1	45.4	55.3	68.3

[a] Sets of four bags were introduced at 2-week intervals. Each figure represents the value for one bag.
[b] Cellulose digestion period in hours.
[c] Nylon bag was lost in the rumen.
[d] Sample accidentally discarded during analysis.

followed so that a longer period of antibiotic feeding could be employed without serious complication of interpretation due to possible environmental effects on apparent cellulose digestion, which might alter values for all animals similarly regardless of treatment.

Results of experiment II are presented in table 2. The percent cellulose digested in 24, 48, 72, and 96 hours was 30.1, 47.6, 63.0, and 67.6, re-

spectively, for the controls, and 28.1, 45.4, 55.3, and 68.3 for the antibiotic-fed animals. As in experiment I there was no significant difference due to treatment (chlortetracycline vs. control) in cellulose digestion as measured by disappearance from the nylon bags. The rate of cellulose digestion was most rapid during the first 24-hour period and declined in succeeding periods. There was no indication in either experiment that cellulose digestion the first week after initiation of antibiotic feeding was appreciably different from values obtained in subsequent weeks of antibiotic feeding. At no time during the course of these studies was there indication that rate of feed consumption or health of the animals was adversely affected by introduction of chlortetracycline into the diet.

The observations in the present study were confined to apparent cellulose digestion in the rumen, which may account for the difference between these data and results of digestion trials conducted by others (Bell *et al.*, 1951; Kesler and Knodt, 1952; Horn *et al.*, 1955). Also, there may be a marked difference in the effect on digestion of crude fiber as compared to digestion of purified cellulose, although observations of Lambert and Jacobson (1956) indicated that chlortetracycline feeding affects *in vitro* digestion of cellulose from a purified source and from alfalfa similarly. The present results would seem to be in accord with those of Bartley *et al.* (1953), Perry *et al.* (1954) and Jacobson *et al.* (1954) who found no apparent harmful effects from feeding chlortetracycline to older animals. Moreover, the present data suggest that artificial rumen studies (Kesler and Knodt, 1952; Lambert and Jacobson, 1956; Lodge *et al.*, 1956; and Hanold *et al.*, 1957), which indicate a reduction in cellulose digestion by chlortetracycline feeding, do not reflect accurately the extent of cellulose digestion in the bovine rumen. It is recognized, however, that the conditions imposed in these experiments may not be entirely typical of normal rumen function. Furthermore, some of the cellulose may have been removed from the bags in an "undigested" state. However, when nylon bags containing cellulose were placed in plastic bags filled with auto claved rumen contents and the plastic bags and contents were kneaded for a considerable period of time, no appreciable loss of cellulose from the nylon bags occurred. This suggests that some change in the cellulose probably took place in the rumen prior to disappearance of the cellulose from the bags. It is possible also that the antibiotic feeding system employed may have reduced the differences between treatments, although no indication of this possibility is apparent from the results obtained when the antibiotic first was introduced.

Summary

Nylon bags containing known quantities of cellulose (Solka-Floc) were introduced through fistulas into the rumens of three steers and cellulose digestion (as estimated by disappearance from the bags) was determined. In the first experiment, two treatments (control vs. chlortetracycline,

approximately 13 mg. per 100 lb. live weight daily) were used alternately for 3-week periods with a total of four control and three antibiotic periods. In the second experiment, the three animals served as controls for 6, 10, and 14 weeks, respectively, preceding a 12-week period of antibiotic feeding at the same level as in experiment I. The final control period was 10, 6, and 2 weeks for the respective animals. Cellulose digestion (48 hours after the cellulose was introduced into the rumen in the first experiment and 24, 48, 72 and 96 hours after cellulose introduction in the second) was not altered appreciably by chlortetracycline feeding.

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AVAILABILITY OF VITAMIN A AND CAROTENE AS INFLUENCED BY SOFT PHOSPHATE[1]

E. S. Erwin and H. M. Page

University of Arizona,[2] Tucson

SOFT phosphate has been reported to be unsatisfactory as a source of phosphorus for chicks, by Gillis *et al.* (1954) and for swine by Chapman *et al.* (1955). On the other hand, Plumlee *et al.* (1955) and Gobble *et al.* (1956) reported similar performance for swine that were fed rations supplemented with either soft phosphate or other types of phosphorus supplements. Working with cattle, Ammerman *et al.* (1957) reported decreased phosphorus retention from soft phosphate, and Long *et al.* (1956) found that phosphorus deficiency symptoms were produced on diets supplemented with soft phosphate.

The incorporation of sodium bentonite, a colloidal clay, into purified rations has resulted in a vitamin A deficiency in rats, as reported by Laughland and Phillips (1954), and in chicks, by Briggs and Spivey (1954). Erwin *et al.* (1957) reported that colloidal clay not only adsorbed carotene but also other pigments found in alfalfa. However, when bentonite was added to a steer ration that contained dehydrated alfalfa, carotene utilization was not altered.

The studies presented in this paper include: (1) The adsorptive affinity of soft phosphate for vitamin A, carotene and alfalfa pigments *in vitro;* (2) the vitamin A utilization, rate of gain and feed utilization by lambs as influenced by source of supplemental phosphorus.

Procedure

The adsorption of beta carotene, crystalline vitamin A and alfalfa pigments by soft phosphate was investigated *in vitro*. Further, an *in vivo* study was conducted with 15 individually penned wether lambs fed rations supplemented with phosphorus from three sources. The wethers were randomly allotted to three treatment groups with five-block replicates, and self-fed for 45 days the all-pelleted low-pigment rations shown in table 1. These three rations were formulated to contain equal amounts of phosphorus and equal amounts of vitamin A; they varied only in source of phosphorus, as follows: (1) Bone meal, (2) soft phosphate and (3) dicalcium phosphate. Liver samples were obtained by biopsy from all wethers initially and at the end of the study, and were subjected to vitamin A analyses as described by Gallup and Hoefer (1946). Feed consumption and

[1] Arizona Agricultural Experiment Station Technical Paper No. 444. Throughout this paper the term "soft phosphate" refers to the commercial feeding grade of soft phosphate with colloidal clay.
[2] Department of Animal Science.

body weight changes were recorded, and all data were subjected to the multiple range test for statistical significance as outlined by Li (1957).

Experimental and Results

Initially, 30.7 mcg. of pure beta carotene in light petroleum ether were thoroughly mixed with varying amounts of soft phosphate. The carotene was completely adsorbed by 0.4 gm. of soft phosphate. The commercial

TABLE 1. PERCENTAGE COMPOSITION OF EXPERIMENTAL RATIONS [a] [b]

Items	Bone meal	Soft phosphate	Dicalcium phosphate
Cotton gin trash	65.0	65.0	65.0
Barley	22.1	21.4	22.3
Molasses	10.0	10.0	10.0
Urea	1.4	1.45	1.46
Trace mineral salt	0.5	0.5	0.5
Bone meal	1.0	—	—
Soft phosphate	—	1.65	—
Dicalcium phosphate [c]	—	—	0.74
Chemical constituents:			
Moisture	6.4	6.72	6.18
Protein	11.31	10.90	10.82
Ether extract	2.39	2.35	2.65
Crude fiber	12.95	15.21	15.05
Ash	9.48	9.23	10.87
Phosphorus	0.15	0.16	0.15

[a] One-half inch pellets.
[b] Vitamin A, furnished through the courtesy of Chas. Pfizer & Co., was added to all rations to approximate 1200 I.U. per lb. of feed.
[c] Contained 19% phosphorus, 39% calcium and 0.19% fluorine.

feeding grade of soft phosphate was separated into particle-size portions and varying amounts of each portion were mixed with petroleum ether solutions of carotene. More carotene was bound as the amount of soft phosphate was increased and as the particle size decreased. This result indicated adsorption of carotene by the soft phosphate. Furthermore, 71% of the carotene in a petroleum ether solution was adsorbed by 0.1 gm. of dried phosphate as compared to 26% by a similar weight of the undried material.

A sample of alfalfa was refluxed with a 30:70 mixture of acetone and petroleum ether for one hour, and 15 ml. aliquots were treated with 0, 0.1, 0.3 and 0.4 gm. of dried soft phosphate. Spectrographic analyses of a 1:10 dilution of the treated extracts revealed that the phosphate bound some pigments; however, chromatographic analyses revealed carotene was not adsorbed by the phosphate in these acetone mixtures. It appears, therefore, that the presence of soft phosphate in mixed feeds would not interfere with conventional carotene analysis. When acetone was removed from the alfalfa extract and the original volume restored with petroleum

ether, a marked adsorption of pigment was observed (figure 1). Further, chromatographic analysis revealed that 0.4 gm. of soft phosphate bound almost all the carotene. These results with soft phosphate are similar to those reported by Erwin *et al.* (1957) with sodium bentonite. Addition of acetone:petroleum ether (30:70) to soft phosphate that had adsorbed pigments released pigment (figure 1). However, the adsorption peak of the released pigments differed from the adsorption peak of the original alfalfa extract, indicating that carotene was not released by the acetone treatment. Chromatographic analyses substantiated those findings.

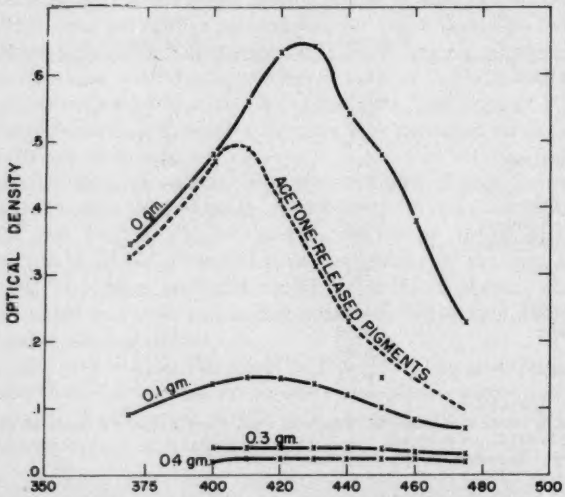

Figure 1. Alfalfa pigment adsorption by soft phosphate in petroleum ether.

When solutions of vitamin A acetate and vitamin A alcohol were treated with soft phosphate, there was a marked loss of vitamin A (figure 2) accompanied by the development of a blue color on the phosphate. When soft phosphate with bound vitamin A was treated with acetone, a stable yellow color appeared in solution that gave no blue color when treated with antimony trichloride. Laughland and Phillips (1954a) reported similar findings when sodium bentonite with adsorbed vitamin A was treated with diethyl ether. Apparently, the colloidal clay that is associated with soft phosphate acts in a manner similar to sodium bentonite relative to pigment adsorption.

Studies with rats and sheep by Klosterman *et al.* (1952) indicated that phosphorus supplementation depressed carotene and vitamin A utilization. However, in the present study supplementation with soft phosphate resulted in a significantly greater loss of vitamin A from the liver ($P<.025$)

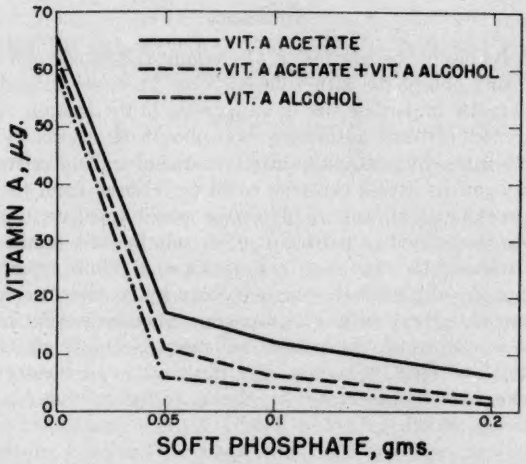

Figure 2. Adsorption of vitamin A alcohol and viamin A acetate by soft phosphate.

of the lambs than did supplementation with either bone meal or dicalcium phosphate (table 2). No significant difference in this respect was found between lambs fed bone meal and dicalcium phosphate. Significantly faster gains were made by the lambs fed dicalcium phosphate ($P<.05$) than by those fed either bone meal or soft phosphate. Soft phosphate, as compared to dicalcium phosphate, significantly ($P<.05$) depressed feed efficiency.

TABLE 2. THE INFLUENCE OF SOURCE OF PHOSPHATE ON FEED-LOT PERFORMANCE AND VITAMIN A UTILIZATION BY WETHER LAMBS

Items	Bone meal	Soft phosphate	Dicalcium phosphate
Number of lambs	5	5	5
Number of days	45	45	45
Body weight change, lb.			
Initial	89.0	93.0	94.3
Final	107.0	107.4	117.7
Daily gain	0.32	0.275	0.524
Feed per lamb per day, lb.	3.58	4.04	4.43
Feed per lb. wt. gain, lb.	11.19	14.69	8.45
Hepatic vitamin A [a]			
Initial content	124.04	159.88	126.82
Final content	82.84	77.52	72.98
Change	—41.20	—82.36	—53.84

[a] Mcg. per gm. of fresh liver.

Summary

In vitro studies of the adsorption of vitamin A, carotene and alfalfa pigments by soft phosphate with colloidal clay gave the following results. Either a decrease in particle size or an increase in the amount of soft phosphate, increased carotene adsorption. Soft phosphate adsorbed all pigments from a petroleum ether extract of alfalfa in the absence of acetone. Nearly all alfalfa pigments except carotene could be released from the soft phosphate by acetone treatment. A blue color was formed on the phosphate when it was treated with a petroleum ether solution of vitamin A; acetone treatment released the blue color and produced a yellow supernatant. The binding capacity of phosphate was influenced by its moisture content.

Soft phosphate, bone meal and dicalcium phosphate were compared as sources of supplemental phosphorus for sheep. Soft phosphate depressed weight gains and feed utilization. The inactivation of dietary vitamin A by soft phosphate was indicated by the magnitude of hepatic vitamin A loss in a 45-day period.

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AN ARTIFICIAL RUMEN TECHNIQUE FOR STUDYING RUMEN DIGESTION *IN VITRO* [1]

L. R. FINA,[2] G. W. TERESA,[2] AND E. E. BARTLEY [3]

Kansas Agricultural Experiment Station, Manhattan

SEVERAL types of artificial rumens have been devised for studying the utilization of nutrients *in vitro*. One of the earliest types was designed by Louw *et al.* (1949). Huhtanen and Gall (1952) developed a miniature artificial rumen which is a simplified adaptation of Louw's apparatus. This miniature artificial rumen is convenient and useful in routine tests of microbiological cellulose digestion *in vitro*. Huhtanen, *et al.* (1954) pointed out the advantages and disadvantages of the miniature artificial rumen, and indicated that the main advantages were convenience and economy. However, with present artificial rumen techniques, it is still difficult to correlate results obtained *in vitro* with those actually obtained in the animal. Several criteria were suggested by Warner (1956) as guides in comparing *in vitro* studies with rumen function. He included ". . . the maintenance of numbers and normal appearance of the bacteria, selenomonads, and protozoa of the rumen; the maintenance of normal rates of digestion of cellulose, starch, and protein; and of normal interactions between these; the ability to predict quantitative results *in vivo*." Warner (1956) also devised a system whereby these criteria were met for about 8 hours; however, after 8 hours, there was increasing failure to meet these conditions. This paper is a report on a new *in vivo* artificial rumen technique which has been developed in an effort more closely to simulate rumen function than do present artificial rumen techniques.

Description of Apparatus. The apparatus used in this work consisted of a porcelain test tube equipped with tubes which permitted free escape of fermentation gases. This *in vivo* artificial rumen (figure 1) was placed in the rumen of a fistulated animal (figue 2). Details of the assembly are shown in figure 3. The porcelain test tube in which the reactions occur was attached to a glass frothing tube and rubber pressure tubing. The porcelain test tube was especially ordered [4] and has a porosity much finer than that used for filtering bacteria. In order to maintain aseptic conditions in the tube a tight rubber sleeve was used to insure that no liquid seeped in. The glass frothing tube, protected by an aluminum shield, was used to prevent liquid from percolating up the pressure tube and thus blocking free escape of gases. Rubber tubing connected to the apparatus and extending from the fistula to a simple manifold permitted free escape of gases.

[1] Contribution No. 334, Dept. of Bacteriology, and No. 258, Dept. of Dairy Husbandry.
[2] Department of Bacteriology.
[3] Department of Dairy Husbandry.
[4] Coors T35 fired to cone 04—Coors Porcelain Company, Golden, Colorado.

Figure 1. Assembled in vivo artificial rumen.

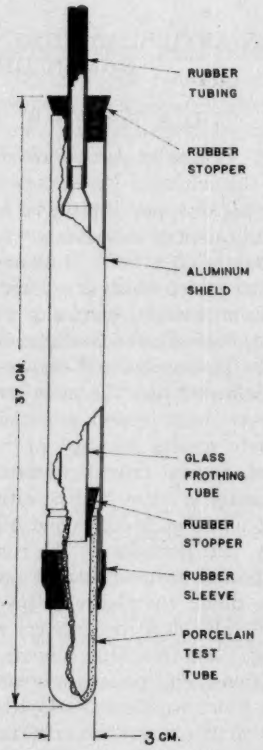

Figure 3. Cut away diagram of the in vivo artificial rumen.

Failure of the gases to escape freely would cause sufficient pressure to develop to force all of the liquid out of the porcelain tube into the rumen. A constant stream of oxygen-free nitrogen was directed through the manifold at a rate of approximately 60 bubbles per minute to sweep out any possible atmospheric oxygen, keep the system at atmospheric pressure, and help maintain anaerobic conditions.

The apparatus was inserted in an upright position directly under the fistula of the animal and against the left wall of the rumen (figure 2). As many as five or six of these assemblies can be placed simultaneously in one fistulated animal. The tubing, allowing for the escape of gases and connecting with the manifold, was kept free from kinking or tangling by a weight and pulley arrangement (figure 2).

Procedure

The permeability characteristics of the porcelain test tubes were checked *in vitro* under various conditions. In all cases before use the tubes were heated for 1 hour in a muffle furnace at 650° C. and dialyzed overnight in tap water to remove any organic matter present.

Repeated tests were also conducted *in vivo* to determine whether bacteria or any other materials would penetrate into the tubes. Whatman's No. 1

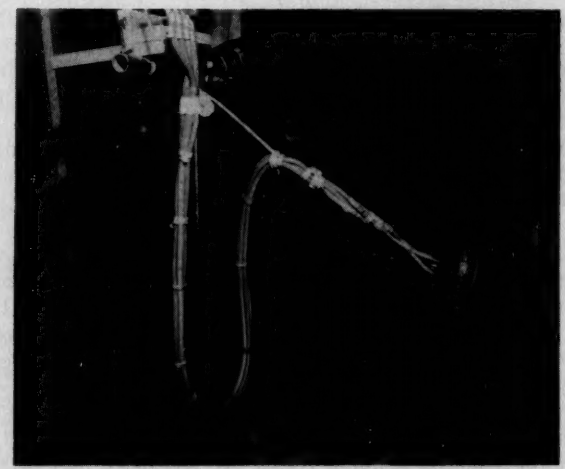

Figure 2. A fistulated animal with in vivo artificial rumen suspended in place and illustrating attachment of escape tubes to manifold.

ashless cellulose powder was sterilized by autoclaving it at 121 ° C. for 30 minutes or longer in the cotton-plugged tubes which had been previously ignited. The tubes were then completely filled with sterile distilled water, left uninoculated, and the cotton plugs replaced with sterile Escher rubber stoppers. To keep the top of the rubber stopper dry and free from possible contamination, a protective rubber gasket, an aluminum shield and a rubber sleeve were used. The tubes were then tied to a nylon line and placed in the rumen of fistulated animals. At various intervals the tubes were removed and contents tested for cellulose decomposition, amount of volatile acids, sterility, and general appearance of the fluid.

One set of four-year-old identical twin fistulated steers was used. The animals were exercised regularly and were fed daily 16 lb. of alfalfa hay and 4 lb. of a 12% protein grain mixture. They were fed at 8 a.m. and 6 p.m. Drinking water was withheld 2 hours before obtaining rumen samples; otherwise, the animals were permitted to drink water at will.

The rumen fluid used as inoculum in the porcelain tubes was obtained by drawing fluid through the fistula and passing it through a metal strainer into a flask under partial vacuum. The flask, still under partial vacuum, was placed in a water bath at 40° C. and after a few minutes the fluid separated into three layers. The heavier material settled to the bottom; the lighter, frothier particles floated to the top leaving a central yellowish layer relatively free of particles. This central layer was used for the inoculum, 35 ml being transferred to the porcelain tube under a stream of nitrogen to insure a minimum of contact with atmospheric oxygen.

Patapar [5] vegetable parchment (250 to 500 mg.) was used in visual estimations of cellulose decomposition. Whatman's No. 1 ashless cellulose powder was used for the quantitative determinations of cellulose decomposition. The method of Crampton and Maynard (1938) slightly modified was used.

The rate of cellulose decomposition was tested by placing tubes containing 500 mg. of cellulose, with and without an inoculum, in the rumen for 10 days. Cellulose estimations were made visually. The experiment was repeated and the digestion of cellulose determined quantitatively.

One of the criteria suggested by Warner (1956) for evaluating artificial rumen function is that ". . . the maintenance of normal rates of digestion of cellulose . . ." should be upheld throughout the experiment. It seems that this criterion might be made more dependable, if, after digestion of the original substrate one adds, without additional inoculum, digestion of subsequent increments of cellulose should proceed continuously. To test this, 250 to 500 mg. of cellulose (Patapar) was added every few days to the *in vivo* artificial rumen which was suspended in the rumen of a steer for 9 days. The *in vivo* artificial rumen was inoculated only once at the start of the experiment and the disappearance of cellulose observed every few days.

This continuous digestion test was repeated and cellulose digestion determined quantitatively. Six tubes were employed and all were placed in the rumen at the same time. The test was arbitrarily terminated at the end of 10 days. Tube 6, the control, containing distilled water was kept in the rumen throughout the test. The tubes were removed at time intervals recorded in table 2. All but one of the porcelain tubes were immediately returned to the rumen after adding new cellulose under a stream of oxygen free nitrogen.

The question whether a small inoculum would digest cellulose with a subsequent increase in microbial flora was answered in part by an experiment in which three tubes, each containing a 10 ml. inoculum and 28 ml. of distilled water, were used. The inoculum was diluted 1:4. The diluted inoculum and 500 mg. Patapar were added and the *in vivo* artificial rumen

[5] Patapar vegetable parchment 27–47. Courtesy of the Paterson Parchment Paper Co., Bristol, Pennsylvania.

placed in the animal. At 0, 24, 48, and 96 hours packed cell volume and remaining cellulose were observed.

Results and Discussion

In vitro studies disclosed that the tubes were permeable to acetic, propionic, butyric, valeric, iso-valeric, and caprioic acids, and substances such as Evan's blue, methyl red, urea, and glucose. The enzyme urease did not penetrate into the tube. More tests are being conducted to determine pore size as related to permeability to various substances.

TABLE 1. TESTS TO DETERMINE PERMEABILITY OF PORCELAIN TEST TUBES TO CERTAIN CONSTITUENTS OF RUMEN FLUID

Time in rumen	Cellulose initial	Cellulose recovered	Volatile fatty acids/ml. inside test tubes	Volatile fatty acids/ml. rumen fluid	Bacterial penetration
days	mg.	mg.	μ moles	μ moles	
1	1000	969	23.52	.....[a]	Sterile
	1000	966	25.54	.....[a]	"
	1000	972	.....[a]	.....[a]	"
2	500	490	105.8	100	"
5	500	527	94.1	97	"
	500	495	115.6	115.6	"
7	500	497	115.6	115.9	"
8	500	510	119.6	111.7	"
12	500	490	115.6	119.6	"
21	500	500	129.6	137.2	"

[a] No determination made. It is assumed that the volatile fatty acid concentration for the first day is approximately the same as on other days.

It was learned that bacteria were unable to penetrate the uninoculated sealed sterile porcelain tubes containing cellulose since no decomposition of cellulose occurred even after 21 days (table 1). Cultural as well as microscopic examinations of the fluid within the tubes revealed no contamination.

Volatile acids diffused into the tubes, and after 24 hours their concentrations inside the tubes closely duplicated the concentrations found in the rumen fluids bathing them. The fluid in the porcelain tube, after 24 hours, became amber colored and appeared crystal clear. Soon after the fluid was transferred into an Erlenmeyer flask, a precipitate of calcium carbonate was formed. The fluid is to be tested for other materials.

In studies on the rate of cellulose decomposition it was found that when using distilled water and no inoculum no cellulose decomposition was observed even after 10 days. In inoculated tubes, 500 mg. of cellulose as Patapar were decomposed completely in less than 48 hours. This observation was confirmed by quantitative experiments in which 80 to 88% of

cellulose in the form of Whatman's No. 1 filter paper was decomposed in inoculated tubes within 48 hours. In two trials, 308 and 341 mg. of cellulose were used. In uninoculated controls in which the same amount of cellulose was used, no cellulose was decomposed. These results are comparable to those obtained by other workers (Cheng, *et al.*, 1955; Dehority, *et al.*, 1957) using microorganisms from 40 ml. of rumen juice. Cheng *et al.* (1955) in their work with washed bacterial suspensions state that "*in vitro* studies may not yield results that are directly applicable to *in vivo*

TABLE 2. CONTINUOUS DIGESTION OF FILTER PAPER USING THE SAME INOCULUM IN THE *IN VIVO* ARTIFICIAL RUMEN

Tube	Amount cellulose added at zero time	Cellulose in 35 ml. rumen fluid inoculum	Time interval in hours indicating quantity of cellulose added and when porcelain tubes were removed						Total cellulose added	Total digestion
			38	76	117	157	198	240		
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	%
1	300	3	R[a]						303	70
2	300	3	200	R					503	82
3	300	3	200	200	R				703	91
4	300	3	200	200	200	200	R		1103	93
5	300	3	200	200	200	200	200	R	1303	88
6 (Control)	300	3	0	0	0	0	0	R	303	(4)[b]

[a] Indicates removal of tubes from rumen.
[b] Within experimental error of method used.

conditions. . ." but they feel that with their method ". . . it is, nevertheless, reasonable to suggest that such may be the necessary conditions that are likely to meet the needs of cellulolytic microorganisms in the rumen of live animals." Their reasoning may be entirely true. The *in vivo* artificial rumen seems to uphold their *in vitro* results.

That an initial inoculum could continuously digest cellulose in the *in vivo* artificial rumen was shown by the complete digestion of 250 to 500 mg. increments of Patapar added periodically over a period of 9 days. When this experiment was repeated (table 2) using quantitative determinations for cellulose, with the use of only one initial inoculum, cellulose digestion continued during a period of at least 10 days.

When diluted inoculum and Patapar were added to the *in vivo* artificial rumen there was a fourfold increase in packed cell volume after 48 hours (table 3). Surprisingly, the final amount of cellulose (Patapar) digestion observed visually was the same as when undiluted rumen fluid was used as the inoculum. The only difference was that a longer time was necessary. Therefore, it would seem that cellulose digestion is dependent upon establishing a certain quantity of cells. The drop in numbers of cells after 48 hours is significant, since autolysis apparently followed when all the cellulose was digested.

It was learned by the use of this apparatus that near anaerobic conditions must prevail throughout the trials in order to obtain maximum

cellulose digestion. The rumen fluid if not collected under anaerobic conditions showed a greatly decreased ability to decompose cellulose. Also if air was permitted to enter the outlet tubing at any time during the trials, cellulose decomposition completely ceased. No explanation of this fact can be offered at this time. It is recognized that this is contrary to the results of others (Anderson *et al.*, 1956; Hall *et al.*, 1955) in which no elaborate measures were made to keep 0_2 away from these cultures.

Occasionally numbers of protozoa were estimated and protozoal activity was found to be sustained for 24 hours, but after 38 hours this activity was greatly reduced.

TABLE 3. CHANGES IN THE QUANTITY OF BACTERIAL CELLS ACCOMPANYING CELLULOSE DIGESTION IN THE *IN VIVO* ARTIFICIAL RUMEN [a]

Total hours tubes suspended in rumen	0	24	48	96
Packed cell volume mm³	22	32	80	negligible [b]
Patapar digestion % (visual determination)	—	trace	85	100

[a] 10 ml. rumen fluid plus 28 ml. distilled water used as inoculum.
[b] Apparent autolysis after all cellulose used.

The main objective of an artificial rumen is to duplicate conditions that occur in the living animal. The *in vivo* technique places the apparatus directly in the rumen. It is assumed that the conditions prevailing within the apparatus are the same as those in the animal. No assumption is made that the living rumen with its many variables such as temperature (Brody *et al.*, 1955) and pH (Bartley, 1955) fluctuations are essential or beneficial to cellulose digestion. The fact remains that they do exist and as such can not be duplicated *in vitro*.

It is believed that this technique should more closely approximate actual rumen conditions than other methods. Its application to many types of determinations in the study of bloat and feed utilization seems possible.

Summary

An *in vivo* artificial rumen technique for studying utilization of cellulose and other nutrients by rumen microorganisms has been developed in an effort more closely to simulate, under controlled conditions, rumen function. The *in vivo* artificial rumen consists of a porcelain test tube attached to a glass frothing tube and gas escape mechanism. The substrates and inocula are placed in the tube and the apparatus suspended in the rumen of a fistulated animal. The tubes were found to be permeable to fatty acids (C_2 to C_6 tested) and glucose but not permeable to bacteria. Using this *in vivo* artificial rumen technique no cellulose was ever decomposed in

non-inoculated tubes placed in the rumen. In inoculated tubes the digestion of 500 mg. of cellulose was completed in about 48 hours. An initial inoculum continuously digested added increments of cellulose at an unabated rate to the extent of about 90% over an extended period of time (10 days). The quantity of the organisms in a diluted inoculum increased during active cellulose digestion.

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ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS IN BEEF CATTLE. I. HERITABILITY OF GROWTH RATE ESTIMATED FROM RESPONSE TO SIRE SELECTION [1]

C. M. KINCAID AND R. C. CARTER

Virginia Agricultural Experiment Station, Blacksburg

AN accurate estimate of heritability is the most important single genetic parameter needed to make effective breeding plans for the improvement of economic characters in farm livestock. Perhaps the most important such character in beef cattle is growth rate and particularly gains in the feedlot or on pasture following weaning. Previous estimates of heritability of postweaning gains reported in the literature and obtained from paternal half-sib analysis have been rather high (in excess of 0.5). It seemed important, therefore, to make some test of those "high" heritability estimates arrived at by indirect means. An experiment to make such a test was started in 1947 at the Virginia Station. The objectives were to measure the heritability of postweaning gain and the progress to be made by selection by comparing the performance of the progeny of bulls that had been themselves selected for rapid and slow rates of gain. The results of this experiment are reported herein.

Experimental Procedure

Each year from 1947–48 to 1952–53 a number of weanling beef bull calves were on performance feeding tests following weaning at the main station at Blacksburg or at the Beef Cattle Research Station at Front Royal. In the first year, 1947–48, eight bulls were fed; 24 were fed the next season, 1948–49, and an average of about 40 bulls each of the succeeding years. In the first 3 years the bulls were borrowed from breeders, mostly in Virginia, but a few from Maryland. Beginning in 1950–51 all bulls were owned by the Virginia Station or the U.S.D.A. Most of these were produced in the purebred herds at the Front Royal Station, but a few were purchased from breeders at weaning time. In the first two years, only Hereford calves were fed, but in the latter years Angus and Shorthorn groups were added to the feeding test.

The bulls were individually full-fed a ration of mixed hay and grain for a feeding period averaging 168 days. The hay and grain were fed separately, free choice, the first four seasons. Individual bulls were observed to differ widely in the relative proportions of hay and grain consumed. Beginning 1951–52 the ration was changed to a mixture of 60%

[1] Contributing to Southern Regional Project S–10.

concentrates and 40% ground hay to insure each animal getting the same proportion of concentrates.

At the end of the feeding test (about April 1) the bulls to be progeny tested were selected, the sole criterion being the rate of gain during the feeding period. An equal number of bulls making the highest gains and those making the lowest gains were retained for progeny testing. Four bulls were selected in 1948, eight in 1949, ten in 1950, eight in 1951, six in 1952, and two in 1953, a total of 38. The 19 bulls selected for high gains averaged 2.24 lb. per day gain during the feeding test while the 19 low gaining bulls averaged 1.65 lb., a difference of 0.59 lb. per day which was more than twice the pooled standard deviation of daily gains (0.28) among bulls within year, breed, and age groups.

The selected bulls were paired (one high-gaining, one low-gaining) within breed year and age groups for the progeny test. Each pair was mated to cows randomized from groups of half-sisters from an unselected herd of grade Herefords, all at the main station at Blacksburg except for one pair of bulls which were mated at the Front Royal station in 1952. The number of cows per bull varied from 10 to 20 in the different years.

The steer progeny or a sample of the steer progeny of each bull was started on feed for record of performance testing immediately after weaning. The weaning weight was taken as the initial weight for the feeding test. Usually four steers per progeny group were fed if this many were available. If more than four were available, a sample of four was chosen at random. In 1952, five steers per progeny were fed out and in 1953 and 1954 all available steers were placed on feed.

Most of the steers were fed in individual stalls so that individual feed consumption records could be kept. The ration consisted of ground ear corn, cottonseed meal and fair quality mixed hay. The hay and grain were fed separately, free choice, the first two years, but in the third and subsequent years a mixture of 60% concentrates and 40% ground hay was self-fed. The length of the feeding period averaged about 200 days. At the end of the feeding period, all the steers were graded individually as slaughter steers and slaughtered at a nearby packing plant. Carcass weights and grades were obtained.

The heifer calves were wintered on roughage with a small amount of concentrates to gain from ¾ to 1 lb. per day. They were grazed on bluegrass-white clover pastures during their yearling summer from about April 20 to October 10. Gains made on pasture constituted the gain evaluation of these heifers. The heifers of the first three calf crops were grazed at Blacksburg on pastures somewhat below average in quality for this area and the resulting gains are considered less than maximum. The last three groups were grazed during their yearling summer at the Southwest Research Station at Glade Spring on bluegrass pastures excellent in qual-

ity and liberal in quantity. The pastures were considered sufficient to insure near maximum gains on pasture.

All heifers were graded as yearling feeders at the end of the grazing season.

Results

Phenotypic Performance. The gains of the selected bulls and of their steer and heifer progenies are shown by sire pairs in table 1. The average daily gain in feedlot of the steer progeny of the 19 sires selected for high gains was 1.81 lb. per day. That of the 19 low gaining sires was 1.71 lb., a difference of 0.10 lb. per day. In three of the sire pairs the steer progeny of the low gaining bull had higher feedlot gains than did the progeny of the high gaining bull. All of these were in the 1951 calf crop. In four other sire pairs the progeny differences were zero or near zero (0.03 lb. per day). Two of these were in 1951.

The heifer progenies were compared on the basis of gains made on pasture during their yearling summer. The average difference between the progenies of the high and low gaining sires was 0.06 lb. per day. The differences, especially during the first three years, were narrower than for the steers full fed in the drylot. In the first two years the heifer progeny of the low gaining bull of two of the six pairs made higher gains than did those of the high sire. The performance of the heifer progenies of the 1951 calf crop was similar to that of the steers in that four of the five progenies of the low gaining sires had higher gains than did those of the high gaining sires, although the differences in two of them were quite small (—.02 and —.04 lb. per day). It seems likely that the somewhat unsatisfactory performance of the heifers during these years may be attributed to the fairly low quality of the pastures. This does not explain the reversal of performance of the 1951 heifer progenies which behaved quite similarly to the steers of the same progenies.

The heifers of the 1952, 1953, and 1954 calf crops were grazed during their yearling summer at the Southwest Station on excellent quality pasture. In these years the average difference between the heifer progenies of the high and low sires was actually larger than for the steers full fed in drylot. This suggests that if pastures are high in quality, comparisons from grazing tests may be as satisfactory for sire evaluation as those from feedlot tests. This would be of considerable advantage in areas where grain is scarce and expensive.

Estimate of Heritability. Heritability was estimated by dividing the weighted average difference between the offspring of the paired high and low gaining sires by the difference between the sires and doubling the resulting regression. This method was used by Lush, Norton and Arnold (1941) to estimate heritability and repeatability of milk and butter fat production. A method of weighting for varying numbers or progeny from

TABLE 1. AVERAGE DAILY GAIN ON TEST FOR SELECTED SIRES AND THEIR STEER AND HEIFER PROGENIES

Year	Sire pairs [a]	Sires			Steer progeny, feedlot					Heifer progeny, pasture				
		Av. daily gain			Number		Av. daily gain			Number		Av. daily gain		
		High	Low	Dif.	High	Low	High	Low	Dif.	High	Low	High	Low	Dif.
1949	H1	2.00	1.76	0.24	4	5	2.24	2.16	0.08	6	3	1.52	1.66	—.14
	H2	1.95	1.76	.19	5	1	2.22	1.73	.49	1	6	1.61	1.49	.12
1950	H3	2.36	1.62	0.74	4	4	1.95	1.82	0.13	4	4	1.41	1.53	—.12
	H4	1.91	1.20	.71	3	4	1.95	1.58	.37	5	8	1.54	1.40	.14
	H5	1.89	1.52	.37	4	4	1.90	1.84	.06	5	4	1.62	1.30	.32
	H6	1.83	1.45	.38	4	3	1.77	1.70	.07	4	7	1.33	1.36	.03
1951	A1	2.16	2.04	0.12	4	4	1.84	1.81	0.03	3	2	1.42	1.44	—.02
	A2	2.23	1.32	.91	3	3	1.90	2.05	—.15	7	5	1.48	1.38	.10
	H7	2.67	1.64	1.03	4	4	1.79	1.76	.03	5	5	1.29	1.33	—.04
	H8	2.02	1.70	.32	4	4	1.60	1.88	—.28	5	2	1.31	1.62	—.31
	SH1	1.95	1.68	.27	4	2	1.91	2.00	—.09	3	7	1.28	1.36	—.08
1952	A3	2.58	1.64	0.94	5	5	1.73	1.73	0.00	10	8	1.80	1.69	0.11
	A4	2.62	1.77	.85	6	5	1.65	1.52	.13	7	5	1.95	1.77	.18
	H9	2.41	1.92	.49	3	2	1.54	1.20	.34	3	7	1.72	1.44	.28
1953	A5	2.38	1.61	0.77	6	10	1.61	1.49	0.12	5	5	1.92	1.60	0.32
	H10	2.30	1.85	.45	8	5	1.64	1.55	.09	4	4	1.86	1.64	.22
	SH2	2.42	1.45	.97	5	7	1.70	1.58	.12	5	5	1.83	1.72	.11
	H11	2.26	1.92	.34	12	10	1.83	1.80	.03	6	5	1.73	1.85	—.12
1954	A6	2.56	1.47	1.09	3	4	1.70	1.38	0.32	8	4	1.27	1.11	0.16
	Total	42.50	31.32	11.18	91	86	34.47	32.58	1.89	96	96	29.89	28.69	1.20
	Average	2.24	1.65	0.59			1.81	1.71	0.10			1.57	1.51	0.06

[a] H1, A1, SH1, etc., indicate Hereford Pair 1, Angus Pair 1, etc.

the individual sires as well as for estimating the reliability of the estimate was worked out by Comstock (1954) suggested to the authors.

The estimate is obtained as

$$b=\frac{\sum_{1} W_1(P_1-P_2)}{\sum_{1} W_1(S_1-S_2)}=\frac{h^2g^2}{2}$$

where b is the regression of offspring on sire, an unbiased estimate of the population parameter β, which is equated on genetic argument to one-half heritability.

P_1 is the average performance of N_1 progeny of the high gaining sire. P_2 is the average performance of N_2 progeny of the low gaining sire. S_1 and S_2 are the performance of the high and low gaining sires, respectively. W_1 is the weight for the i th sire pair. h^2 g^2 is heritability in the narrow sense (Lush, 1946).

The variance of the estimate is

$$\sigma_b^2=\frac{\sum_{1} W_1^2V_1}{(\sum W_1D_1)^2}$$

where D_1 is the difference in performance of the i th sire pair, i.e., $D_1=(S_1-S_2)_1$. V_1 is the variance of the progeny difference from linear regression on D_1.

It can be shown (Comstock, 1954) that optimum weights for a particular sire pair, optimum in the sense of minimizing the variance of the estimate, are obtained by dividing the difference in performance of the paired sires by the variance of the progeny groups.

$$W_1=D_1/V_1$$

These weights were used to obtain separate estimates of regression in the steer and heifer progenies. Two other commonly used systems of weighting for unequal numbers, the inverse of the variance, $1/V_1$, and the ratio N_1 $N_2/(N_1+N_2)$ where N_1 and N_2 are the numbers in the two progenies of a sire pair were also used. Finally an estimate was obtained using the sire and progeny differences unweighted, i.e., $W_1=1$.

These different weights will be referred to as W_1, W_2, W_3, and W_o. Estimates of regression and their variance using the different weighting factors are shown in table 2.

It is standard practice to estimate heritability from parent offspring comparison by multiplying the regression by two (i.e., dividing by ½). However, the validity of the regression method for estimating heritability depends on the variance being equal in the populations from which the data are obtained. This is clearly not the case with the present data. The pooled standard deviation of daily gain for the entire group of bulls, calculated from the variance within year, age and breed groups was

TABLE 2. ESTIMATES OF REGRESSION AND THE VARIANCE OF REGRESSION USING DIFFERENT WEIGHTING FACTORS

Weighting factors	Steers		Heifers	
	b	σ_b^2	b	σ_b^2
$W_1=D_1/V_1$	0.130	$.066\sigma^2$ [a]	0.129	$.057\sigma^2$
$W_2=1/V_1$	.135	$.087\sigma^2$	.122	$.071\sigma^2$
$W_3=N_1 N_2/(N_1+N_2)$	.143	$.086\sigma^2$	.122	$.068\sigma^2$
$W_0=1$ (equal weighting)	.169	$.097\sigma^2$	.107	$.091\sigma^2$

[a] σ^2 is the within sire progeny variance and is 0.0378 for the steers and 0.0389 for the heifers.

0.282 lb. The respective standard deviations within sire progenies was 0.194 for the steers and 0.197 for the heifers. It was, therefore, necessary to adjust the estimates for differences in the variances. This was done by multiplying the heritability estimate, h^2 g^2, by the ratio of the standard deviations (σ_s/σ_p) where σ_s is the within group standard deviation of daily gain for the bulls and σ_p is the within sire progeny standard deviation. The estimate in effect becomes a correlation instead of a regression. Estimates of heritability with and without adjustment for differences in variance for each of the weighting systems are shown in table 3.

Fiducial limits were obtained for the heritability estimates using the variance of the regression. Upper and lower 95% limits for the estimates of h^2 g^2 for steers and heifers using the four weighting schemes are also shown in table 3.

The lowest sampling variance is for the estimate obtained by using weights proportional to the sire difference ($W_1=D_1/V_1$) as was predicted in the model. Equal weighting gave the highest variance with the other two weighting schemes intermediate and fairly close in magnitude of variance. It appears that if the linear model is accepted it is more important to take into consideration the sire differences (D_1) than the variance (V_1).

TABLE 3. HERITABILITY ESTIMATES WITH 95% FIDUCIAL LIMITS FOR DIFFERENT WEIGHTING SYSTEMS

Weights	Heritability (Unadjusted)	95% fiducial limits		Heritability adjustment for differences in variance	95% fiducial limits	
		Upper	Lower		Upper	Lower
		Steers				
$W_1=D_1/V_1$	0.26	0.47	0.10	0.38	0.68	0.14
$W_2=1/V_1$	.27	.50	.04	.39	.72	.06
$W_3=N_1 N_2/(N_1+N_2)$	.29	.52	.06	.42	.75	.10
$W_0=1$	.34	.59	.10	.49	.85	.15
		Heifers				
$W_1=D_1 V_1$	0.26	.044	0.08	0.37	0.64	0.11
$W_2=1/V_1$	.24	.46	.04	.35	.65	.06
$W_3=N_1 N_2/(N_1+N_2)$	.24	.45	.04	.35	.64	.06
$W_0=1$	.21	.46	>0	.31	.65	>0

It remains to inquire which method gives the best estimate of regression and heritability. Although weighting in proportion to the difference between sires, D_1, gave optimum estimates in the statistical sense in that the variance of the estimate is minimized, the authors have intuitive objection to accepting weights based on the difference in sire performance. Perhaps this objection is based on the several cases in the present study where large sire differences gave small or negative differences between the progenies. It may be that the largest sire differences were due to the low sire of a pair being unusually low in rate of gain rather than the high sire having an unusually high rate of gain. It is suggested that unusually low rates of gain may be due to unusual or accidental non-genetic causes, such as sub-clinical infection, rather than to genotypes for very low growth rates. This would imply that there are two classes of environmental effects, one being of considerably larger magnitude than the other. If this were true one would wish to minimize the effect of the very wide sire differences in estimating heritabilities that are to be used to predict progress from selection among sires that are average or better in performance.

In statistical terms, one is throwing doubt on the validity of the linear model. If one accepts the theory that large sire differences are less heritable than smaller ones, then one is suggesting that in fact the relationship is curvilinear instead of linear. The regression line would be of decreasing slope as the sire differences increase. A test of this idea was suggested by Comstock (1954). It is a test of the linearity of the regression of progeny differences on sire differences. The deviation of quadratic from linear was found to be significant at the 5% level and near significant at the 1% level. This strongly suggests that the regression is in fact curvilinear and that large sire differences are less heritable than smaller ones. If this is accepted, the choice of an estimator of regression becomes a different matter from the usually accepted criterion of minimum variance. One would wish some sort of average value of regression rather than minimum variance of the estimate. One of weighting systems in which the sire differences are ignored, such as $W_2 = 1/V_1$ or $W_3 = \frac{N_1 N_2}{(N_1+N_2)}$ might be more useful in predicting progress from selection. The authors prefer one of these or similar ones rather than one based on sire differences or an estimate from the data unweighted.

Discussion. The estimates of heritability of postweaning gain of steers in the feedlot found in this study are considerably lower than most such estimates reported by other investigators. Most of these were derived from paternal half-sib analysis and ranged in value from 0.5 to above 1.00 (Knapp and Nordskog, 1946; Knapp and Clark, 1950; Patterson *et al.*, 1949, 1955; Shelby *et al.*, 1955). The estimates of Warwick and Cartwright (1955) also based on half-sib correlations, but making use of the "gain ratio" rather than absolute values of gain are somewhat lower.

The estimates of heritability of yearling summer pasture gains of heifers found in this study are closer to those reported by other investigators, including Cartwright (1955) and Koch and Clark (1955) ranging from 0.18 to 0.39.

It has frequently been pointed out that the sampling errors of the paternal half-sib method of estimating heritability are high. The sire component of variance is multiplied by a factor of 4 so that any errors in the estimate of the sire component are similarly inflated. Any environmental effects that are peculiar to a particular sire progeny are included in the sire component and multiplied by 4. Also sires that were themselves high gainers, but which sired uniformly low gaining progeny, or vice versa, as was the case with certain sires in this study, would contribute positively to the heritability estimated.

On the other hand, estimates based on regression of offspring on parent, multiplied only by a factor of two, should have lower sampling error and should be less affected by environmental correlations. Most estimates based on regression of offspring on parent, or on results achieved from selection, have been lower than estimates from the same data derived by the half-sib method. An example is furnished by the estimates recently published by Koch and Clark (1955a, 1955b). Estimates were generally lower when derived from regression of offspring on sire or dam than from half-sib correlations.

For these reasons it is concluded that previously reported heritability estimates in excess of 0.5 obtained from half-sib analysis are probably too high. Estimates obtained in the present study from the results of sire selection range from 0.21 to 0.34 unadjusted and from 0.31 to 0.49 adjusted for differences in variance of progeny and sires. These seem more realistic. They are within the range of heritabilities of postweaning growth reported by several investigators in dairy cattle and other species (Craft, 1950; Rendel, 1950; Rae, 1957). However, the confidence limits of the present estimates are rather wide. On the other hand, if the regression of progeny averages on sire differences is curvilinear rather than linear, then the estimates in the present study may be somewhat too low particularly where weighting was proportional to D, the sire differences. The authors, therefore, concluded that the true value of heritability of postweaning gains is positive, that it lies between zero and 0.5 and is probably in the neighborhood of one third.

No explanation is apparent for the several cases in which the differences between progenies were the reverse of those between their sires This was particularly true in the 1951 calf crop. It does suggest, however, that from one fourth to one third of sires selected because their own gains were above average may be expected to sire calves average or below in performance. This would make progeny testing of the selected sires desirable for optimum progress.

Summary

Nineteen high-gaining bulls averaging 2.24 lb. per day and 19 low-gaining bulls averaging 1.65 lb. were selected from the 131 on test over a six-year period. The difference, 0.59 lb. per day, is slightly more than two standard deviations. The selected bulls, 22 Hereford, 12 Angus and 4 Shorthorn, were paired by breed and mated at random to equal numbers of grade Hereford cows for the progeny test. The steer progeny, totalling 192 head, were full-fed for 200 days following weaning. The heifers, 196 head, were wintered largely on roughage and were tested by gains on pasture their yearling summer.

Differences between the averages of the progenies of the high and low gaining sires were 0.1 lb. per day for the steer and 0.06 lb. for the heifers. Heritability estimates based on these differences ranged from 0.26 to 0.34 for the steers and from 0.21 to 0.26 for the heifers, depending on the weights used to adjust for differences in progeny numbers. When adjusted for differences in variance among the sires and within the progenies, i.e., when converted to standard measure, these estimates became 0.38 to 0.49 for the steers and 0.31 to 0.35 for the heifers.

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THE ROLE OF THE CAECUM IN NUTRIENT UTILIZATION BY THE PIG

L. E. Lloyd, D. G. Dale and E. W. Crampton [1]

Macdonald College (McGill University), Quebec, Canada

ALL herbivorus mammals require a roomy compartment in the digestive tract for the maceration, fermentation and solution of the fibrous portions of their food. In ruminants, such a compartment is provided mainly by the rumen. In simple-stomached herbivores (i.e., the horse, rabbit and guinea pig), the large caecum and the colon meet this requirement. The most important function of the caecum in animals of this type has been considered to be that of a reservoir in which fibrous material, as well as other food constituents, can undergo digestion through the action of the contained microorganisms.

Most fowls possess two caeca, and considerable attention has been directed to the physiology of the caeca of the chicken. Browne (1922) regarded the caeca as playing an unimportant part in digestion; in his opinion, they are mainly for absorptive purposes. On the other hand, the work of Radeff (1928), and Mangold (1929) suggests that they are concerned in the digestion of cellulose.

In the pig, the caecum is of significant proportions, being approximately 8 to 12 in. long and 3 to 4 in. wide. Using a caecal fistula, Trautmann and Asher (1941, 1942) provided evidence of cellulose digestion in the caecum of the pig. Nasr (1950) drew attention to the breakdown of raw potato starch by microorganisms in the pig caecum. However, experimental evidence of digestion in the caecum of the pig, in terms of digestibility data, appears to be lacking.

The experiment to be described was designed, therefore, to study the extent to which various ration constituents are digested in the caecum of the pig.

Caecectomy

The extent to which various ration entities are digested in the caecum was studied by determining differences in the digestibility of the constituents of identical rations fed to normal and caecectomized pigs. The caecum was removed when the animals were between 6 and 7 weeks of age. The operative procedure was as follows:

Feed was withheld from the animals for approximately 18 hours prior to the operation. Anesthesia was accomplished by the intravenous in-

[1] Departments of Nutrition and Animal Pathology. Contribution from the Faculty of Agriculture. Journal Series No. 420.

jection of pentobarbital sodium, given to effect, into the marginal ear vein. The animal was secured in dorsal recumbency and the abdomen prepared by washing with a quaternary ammonium solution, drying and painting with tincture of iodine. The general principles of surgical asceptic technique were followed as closely as was considered necessary. A left abdominal incision, 3 in. in length and approximately 1 in. lateral to the mid line was made commencing anteriorly at a point midway between the umbilicus and the pubis. The caecum was located, brought through the abdominal incision and packed off with gauze sponges moistened with sterile physiological saline. The ileo-caecal artery was ligated at the base of the caecum and the mesocolon divided to free the caecum from its mesenteric attachment. Two Carmalt forceps were placed close together at the base of the organ and the tissue between them was divided with a scalpel. The caecal stump was closed using the Parker Kerr method of suturing. The incision through the muscle and peritoneum was closed with a continuous suture of number 1 chromic catgut. The skin was closed with simple interrupted stitches of size zero monofilament nylon. Post operative care consisted of one intra-muscular injection of 400,000 units of procaine penicillin and 0.5 gram of streptomycin given immediately after the operation and the oral administration of terramycin for 3 days post operatively.

Trial 1

Experimental. Four male Yorkshire litter mates were weaned at 2 weeks of age and fed on an early-weaning pig ration. At 6 weeks of age two of these pigs were caecectomized. Exactly 14 days following caecectomy, each of these animals, along with its control, was fed Ration No. 1. All pigs were individually penned throughout the experimental period.

The following rations were fed in the following order to the four pigs: No. 1, high protein, high fiber; No. 2, low protein, low fiber; No. 3, high protein, low fiber; and No. 4, low protein, high fiber; i.e., Replicate 1. Following this, and in order to examine the possibility of the digestive tract becoming adapted to the absence of the caecum insofar as digestive ability is concerned, the four rations were fed once again in the same order (Replicate 2), thus providing a time interval between the feeding of each ration.

After the completion of the second feeding of Ration No. 4, the following rations were fed in the following order to all pigs: No. 5, 5% added fat; No. 6, 10% added fat; and No. 7, 15% added fat.

The composition of the rations is given in table 1.

All rations were fed *ad libitum* for a period of 12 days. During the last 5 days of this period feces samples from each pig on each ration were collected daily, dried, composited for the 5 days and ground for analysis.

The pigs were weighed at the beginning and at the end of each feeding period. Feed and feces samples were analyzed for crude protein, ether

TABLE 1. PHYSICAL AND CHEMICAL COMPOSITION OF RATIONS FED IN TRIAL 1

Ration ingredient	Ration number						
	1	2	3	4	5	6	7
	%	%	%	%	%	%	%
Wheat	62.0	37.0	37.0	—	72.0	64.0	56.0
Oats	—	—	—	26.0	—	—	—
Cereal grass	20.0	—	—	20.0	10.0	11.0	12.0
Corn starch	—	46.5	34.0	41.0	—	—	—
Casein	—	—	7.0	—	—	—	—
Brewers' dried yeast	5.0	5.0	5.0	2.5	5.0	5.0	5.0
Linseed oilmeal	2.5	1.0	2.5	1.0	2.5	3.5	4.5
Fishmeal	2.5	1.0	5.0	1.0	2.5	3.5	4.5
Molasses	5.0	5.0	5.0	5.0	—	—	—
Corn oil	—	1.5	1.5	0.5	5.0	10.0	15.0
20/FOS [a]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Iodized salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ferrous sulfate	8 gm.	8 gm.	8 gm.	8 gm.	8 gm.	8 gm.	8 gm.
Calculated nutrient content							
Protein	17.5	8.7	17.5	8.7	17.0	17.0	17.0
Ether extract	2.4	2.5	2.6	2.6	7.2	12.1	17.1
Crude fiber	7.0	1.7	1.8	7.0	5.2	5.2	5.2

[a] A calcium and phosphorus supplement manufactured by International Minerals and Chemical Corp., Chicago, Ill.

extract, crude fiber, ash and moisture by standard A.O.A.C. procedures. Nitrogen-free extract was determined "by difference". The perchloric acid method of Bolin *et al.* (1952) was used for determining chromic oxide.

As outlined by Schürch *et al.* (1952), the apparent digestibility by each pig of the dry matter, crude protein, ether extract, crude fiber and nitrogen-free extract was determined by the Cr_2O_3 method at two time-intervals for Ration Nos. 1 to 4, but only once for Ration Nos. 5 to 7.

Results and discussion. Surgical removal of the caecum of both pigs was successfully accomplished. Caecectomy had no apparent ill effect on the well-being of the animals, a fact reflected in identical mean growth rates of 1.2 lb. per day for both caecectomized and normal pigs over the experimental period of 20 weeks.

The average apparent digestion coefficients of various constituents of the experimental rations which varied in protein and fiber level, are shown in table 2 for the normal and caecectomized pigs. It should be noted here that the digestion coefficients for Replicates 1 and 2 have been averaged together in all cases, because any effect on digestibility due to replication was similar for both the normal and caecectomized animals. For example,

the apparent digestibility of dry matter, crude protein and ether extract was significantly higher in Replicate 2, but this was true for both categories of pigs. Thus the effect was one of age, and adaptation of the digestive tract to the absence of the caecum was not a factor in any instance.

Upon studying the data in table 2, the general observation may be made that the removal of the caecum from the pig resulted in a slight decrease in the apparent digestibility of all ration constituents studied with the

TABLE 2. THE EFFECT OF CAECECTOMY ON THE APPARENT DIGESTIBILITY BY PIGS OF VARIOUS CONSTITUENTS OF EXPERIMENTAL RATIONS VARYING IN PROTEIN AND FIBER LEVEL

		Mean apparent digestion coefficients [a]				
		Ration 1	Ration 2	Ration 3	Ration 4	
Ration constituent	Caecum	High protein High fiber	Low protein Low fiber	High protein Low fiber	Low protein High fiber	All rations
Dry matter	Present	72 *	89	88	78	82
	Removed	69	88	87	76	80
Protein	Present	67	76	87	59	72 *
	Removed	63	73	86	56	69
Ether extract	Present	13	26	63	56 *	40
	Removed	17	23	65	62	42
Crude fiber	Present	15	12	15	30 *	18
	Removed	13	5	14	16	12
Nitrogen-free extract	Present	83 *	95	94	88	90 *
	Removed	81	95	94	87	89

[a] Each value within each ration represents the mean for two pigs fed in two replicates.
* Difference between means statistically significant at P=0.05.

exception of ether extract. Of particular interest, originally, was the effect of caecectomy on crude fiber digestibility. While there was a tendency for normal pigs to digest crude fiber more efficiently than caecectomized pigs, only in the case of the low-protein, high-fiber ration did the difference attain statistical significance. Perhaps a result of the slightly higher digestion coefficients for crude fiber by normal pigs, was a comparable increase in the apparent digestibility of protein—an increase which attained statistical significance only when all rations were considered together.

While differences in the apparent digestibility of most ration constituents appeared to be in favor of the normal animals, it should be kept in mind that even when statistical significance was reached, the differences were not of practical significance, being well within the range of normal population variability for each of the ration constituents studied.

In table 3 is shown the average apparent digestibility by normal and caecectomized pigs of various constituents of the experimental rations which varied in fat level. Once again, it was observed that normal pigs tended to digest most ration constituents slightly better than caecectomized pigs. However, high levels of added fat were equally well digested by both categories of animals. In these rations, where the crude fiber level

TABLE 3. THE EFFECT OF CAECECTOMY ON THE APPARENT DIGESTIBILITY BY PIGS OF VARIOUS CONSTITUENTS OF EXPERIMENTAL RATIONS VARYING IN FAT LEVEL

		Mean apparent digestion coefficients [a]			
		Ration 5	Ration 6	Ration 7	
Ration constituent	Caecum	5% Added corn oil	10% Added corn oil	15% Added corn oil	All rations
Dry matter	Present	79	77 *	80	79
	Removed	78	73	79	77
Protein	Present	79	76 *	80	78 *
	Removed	77	71	79	76
Ether extract	Present	77	84	87	83
	Removed	78	83	85	82
Crude fiber	Present	18	20 *	40	26
	Removed	15	3	36	18
Nitrogen-free extract	Present	87	85	87	86
	Removed	87	82	86	85

[a] Each value within each ration represents the mean for two pigs.
* Difference between means statistically significant at P=0.05.

was held constant at about 5%, there was again a tendency for normal pigs to digest more fiber than their caecectomized litter mates; the difference attained statistical significance in the case of Ration 6, and when all rations were considered together. Accompanying the increase in fiber digestibility was a slight but significant increase in protein digestibility by the normal pigs when all rations were considered. As in the case of Rations 1 to 4, it must be realized that the observed differences were not of practical significance.

Trial 2

Experimental. Six Yorkshire litter mates (three males and three females) were weaned at 2 weeks of age and fed an early-weaning pig ration. At 7 weeks of age, one male and two females were caecectomized. Exactly 12 days following caecectomy, each of the 6 pigs was fed a commercial hog starter ration. All pigs were individually penned throughout the experimental period.

The commercial hog starter ration was fed for a period of 2 weeks. This was followed by a commercial hog grower ration until approximately 100 lb. liveweight, and from then to market the animals were fed a commercial hog finisher ration. The relevant chemical composition of these rations is given in table 4.

All rations were fed *ad libitum*. During the last 5 days on the starter ration, feces samples from each pig were collected as described in trial 1.

TABLE 4. CHEMICAL COMPOSITION OF RATIONS FED IN TRIAL 2

Ration constituent	Ration		
	Starter	Grower	Finisher
	%	%	%
Protein	19.1	16.4	13.9
Ether extract	3.7	2.9	2.4
Crude fiber	3.8	4.8	5.0
Nitrogen-free extract	53.2	56.8	60.3

In addition, feces were collected in the same way during the second and final week of feeding of each of the grower and finisher rations.

All pigs were weighed weekly. The apparent digestibility by each pig of the dry matter, crude protein, ether extract, crude fiber and nitrogen-free extract was determined by the Cr_2O_3 method once for the starter ration, and at two time-intervals for both the grower and finisher rations.

Results and Discussion. Surgical removal of the caecum of the three pigs was successfully accomplished. As in Trial 1, caecectomy had no apparent adverse effect on the well-being of the animals, since once more identical mean growth rates (1.4 lb. per day over the experimental period of 15 weeks) were observed for both caecectomized and normal pigs.

The average apparent digestion coefficients of various constituents of the practical hog rations are shown in table 5 for the normal and caecectomized pigs. Again it is obvious that caecum removal from the pig has little effect on the animal's ability to carry out normal digestion. Only in the case of the digestibility of the crude fiber of the starter and grower rations were significant difference observed, but, the trend was opposite for the two rations. When all rations were considered together, differences in crude fiber digestibility disappeared.

General Discussion

Caecectomized pigs, whether fed experimental or practical type rations, were capable of maintaining a growth rate identical to that of pigs whose digestive tracts were intact. As a parallel to this observation, Griffith (1935) found that young rats will grow normally for at least 100 days after removal of the caecum. In addition, Herndon and Hove

TABLE 5. THE EFFECT OF CAECECTOMY ON THE APPARENT DIGESTIBILITY BY PIGS OF VARIOUS CONSTITUENTS OF PRACTICAL RATIONS FED FROM WEANING TO MARKET WEIGHT

Ration constituent	Caecum	Mean apparent digestion coefficients [a]					
		Starter (40 lb.)[b]	Grower (56 lb.)	Grower (97 lb.)	Finisher (108 lb.)	Finisher (175 lb.)	All rations
Dry matter	Present	67	76	71	70	70	71
	Removed	68	73	69	70	72	70
Protein	Present	69	76	78	74	77	75
	Removed	70	74	76	75	77	75
Ether extract	Present	69	70	85	68	81	75
	Removed	64	68	85	63	80	72
Crude fiber	Present	5*	41*	12*	3	1	12
	Removed	14	32	2	1	8	11
Nitrogen-free extract	Present	74	83	78	79	76	78
	Removed	76	81	77	79	77	78

[a] Each value within each ration represents the mean for three pigs.
[b] Figures in parentheses indicate average weight of pigs at beginning of digestion trial.
* Difference between means statistically significant at P=0.05.

(1955) showed that the growth rate of caecectomized rabbits was only slightly lower than normal. It has been shown by a number of workers that the caeca of fowls are not essential structures, since they can be removed or occluded without harmful effect to the bird (Durant, 1929; Hunter *et al.*, 1930; Schlotthauer *et al.*, 1933 and Richardson, 1939).

The lack of differences of any appreciable magnitude in the ability of normal and caecectomized pigs to digest the various ration constituents studied was of particular interest. Because of the work of Trautmann and Asher (1941, 1942), it would appear logical that caecectomy might depress crude fiber, and hence indirectly protein, digestibility. Although a trend in this direction was indicated when the non-practical rations were fed, the observed differences were of an insignificant magnitude in terms of practical considerations. On the other hand, the results of Herndon and Hove (1955) support our observations. These investigators found that the

digestibility of crude fiber was equal in both normal and caecectomized rabbits, and that dry matter, protein and fat digestibility were only slightly lower when the caecum was removed. If the caecum in an herbivorous non-ruminant does not contribute appreciably towards digestion, it is unlikely that the caecum of the pig should play a significant role in this process.

Our studies indicate only that the caecum of the pig does not contribute appreciably to the general breakdown of food substances, not that it has no physiological function. Perhaps a more important function of the caecum is in the synthesis of B vitamins. Indirect evidence of such a function has been provided by Taylor *et al.* (1942) and Bénard *et al.* (1946) for rats, by Couch *et al.* (1949) for fowl, and by Nasr (1950) for pigs. Our work cannot clarify this possibility however, since the rations fed were not deficient in the vitamin B complex.

Summary

Caecectomy had no deleterious effect on the growth rate of pigs fed either experimental or practical type rations. Differences in the ability of normal and caecectomized animals to digest various ration constituents were of small magnitude. It was concluded that the caecum plays an insignificant role in the breakdown of food substances by the pig.

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SPECIFIC GRAVITY AS AN OBJECTIVE MEASURE OF MARBLING[1]

L. E. Orme, A. M. Pearson, L. J. Bratzler and W. T. Magee[2]
Michigan Agricultural Experiment Station,[3] East Lansing

THE amount of marbling, or intramuscular fat, is the most important component of quality in grade specifications for beef carcasses. In the past, the amount of intramuscular fat has been evaluated entirely on a subjective basis. Therefore, an objective measure of this attribute would be extremely useful. A simple inexpensive method of measuring marbling, which would maintain the meat in a saleable condition would be most desirable. Specific gravity has been used quite extensively with pork and has proven itself as a reliable measure of carcass fatness. Brown *et al.* (1951), Whiteman *et al.* (1953), Pearson *et al.* (1956) and Price *et al.* (1957) have reported the use and validity of specific gravity as a measure of leanness of various wholesale cuts and pork carcasses. Its use with beef has been restricted. Breidenstein *et al.* (1955), working with beef ribs that varied from high Good to low Prime in grade, used specific gravity as an objective measure of marbling in the rib eye. They stated that little relationship existed between subjectively measured marbling and specific gravity, while a positive correlation existed between subjectively evaluated marbling and ether extract of the rib eye.

This study was undertaken to explore further the usefulness of specific gravity as an objective measure of marbling.

Materials and Methods

Experimental Ribs. Fifty-one wholesale beef ribs were used. Steers from the Michigan State University performance testing program provided 31 ribs, and 20 additional ribs were purchased to add numbers to the study. The ribs obtained from steers on the performance testing program came from 8 Angus and 23 Hereford steers slaughtered in the University abattoir. Following ribbing, the carcasses were graded by a USDA grader to the nearest one-third grade as 2 being Prime, 25 being Choice and 4 Good. The carcasses were aged for 8 days under uniform conditions and cut according to the procedure described by Wellington (1953). The average weight of the ribs from this group of steers was 25.0 lb. The 9–10–11 rib sections were removed from the wholesale rib as described by Hankins and Howe (1946). The *Longissimus dorsi* muscle was excised from the 9–10–11 rib and all external fat removed.

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[2] This paper reports a portion of the Ph.D. thesis to be presented by the senior author to the school for Advanced Graduate Studies at Michigan State University.
[3] Department of Animal Husbandry.

The remaining 20 ribs were obtained from two large packers and came from carcasses that were USDA graded without ribbing. Half of the ribs were Prime and half were Good. The ribs were selected to represent the entire range of each grade. Average weights were 31.1 and 24.3 lb. for the Prime and Good grades, respectively. A portion of the aging period was spent en route from the slaughterer, but otherwise the ribs were handled in the same manner as described previously.

Specific Gravity. The excised ribeye *(Longissimus dorsi)* portion of the 9–10–11 rib section was weighed to the nearest 0.1 gm. in air and to the nearest 0.01 gm. in distilled water. The first 16 ribeyes were weighed in water at room temperature, while all subsequent weighing was at 6° C.

TABLE 1. MEAN AND RANGE FOR VARIOUS MEASURES OF BEEF BY GRADES (51 STEERS)

Measures	Prime		Choice		Good	
	Mean	Range	Mean	Range	Mean	Range
Specific gravity	1.059	1.051– 1.065	1.064	1.057– 1.069	1.065	1.060– 1.071
Percent fat	8.59	6.67 –11.21	5.62	3.49 – 7.84	4.25	1.90 – 7.18
Percent water	69.65	67.57 –71.13	72.40	70.21 –74.14	73.57	71.14 –75.05
Percent protein	21.49	19.85 –22.37	21.18	20.31 –21.92	21.59	20.87 –22.38

Specific gravity was calculated according to the formula given by Brown *et al.* (1951).

Grinding and Chemical Analysis. After weighing in water, the *Longissimus dorsi* muscle was blotted dry and immediately ground 5 times through a 5/64 in. grinder plate. The ground sample was placed in a glass jar, sealed and frozen for subsequent analysis. Water and protein were determined according to the procedure of Benne *et al.* (1956). Ether extract was determined on the moisture-free sample, which was dried in a disposable aluminum weighing dish.

Statistical Analysis. Correlation and regression coefficients were calculated for specific gravity with percentage moisture, protein and ether extract. The correlation coefficients were determined for the 31 ribeyes from the University steers on a within year basis. The additional ribeyes were not used in calculating the correlations since they had been selected on a weight and grade basis and would introduce bias in the results. However, all ribs were used in calculating the regression equation.

Results and Discussion

Specific gravity and percent water of the *Longissimus dorsi* muscle were inversely related to grade (table 1). Conversely, percent fat increased as grade increased. Percent protein showed no definite pattern, as the mean values for all groups were almost equal. The range for specific

gravity, irrespective of grade, was from 1.051 to 1.071 or a spread of only 0.02 unit. This indicates the necessity of minimizing the variance due to experimental methods and procedures and the need of using a balance of the required sensitivity. When grouped according to grade, there was a tendency for specific gravity ranges to overlap somewhat from one grade to another. This would be expected since factors other than marbling are considered in determining a grade for a particular carcass. However, the 31 ribs from the University cattle were graded in a ribbed condition, where marbling could be visually appraised. Since these cattle were uniform in conformation and age, it is probable that marbling was the major factor in determination of grade.

TABLE 2. CORRELATION COEFFICIENTS OF SPECIFIC GRAVITY WITH CHEMICAL ANALYSIS AND GRADE (31 STEERS)

Items	Specific gravity
Percent fat	—.81
Percent water	0.74
Percent protein	0.68
Grade to 1/3	—.68

Fat from the 51 ribs ranged from 1.90 to 11.21%, or a difference of 9.31%. The range was from 67.57 to 75.05% for water, and from 19.85 to 22.38% for protein. Thus, the maximum variation was 7.48% for water and 2.53% for protein. Observation of table 1 shows overlapping of these measures between grades as was shown with specific gravity, but in each of these three items the degree of overlapping was more pronounced between the Choice and Good grades than between the Prime and Choice grades.

The correlation coefficients for specific gravity with percent fat, water, protein and grade to the nearest one-third are listed in table 2. All correlations were highly significant. The highest relationship existed between specific gravity and percent fat with a correlation coefficient of —.81. The correlation between percent water and specific gravity was 0.74. These correlations indicate the validity of specific gravity as an objective measure of total marbling. Percent protein and grade were related to specific gravity with correlations of 0.68 and —.68, respectively. Thus, the variation in specific gravity was associated with 46% of the variation in either percent protein or grade, 50% with percentage water and 66% with percentage fat. As expected, both percent fat and grade (to the nearest one-third) showed an inverse relationship to specific gravity.

Data from all 51 ribs were used to plot regression lines. Figure 1 shows the relationship between specific gravity and percent fat. The regression equation was $Y=445.24-413.30X$, with a standard error of the estimate

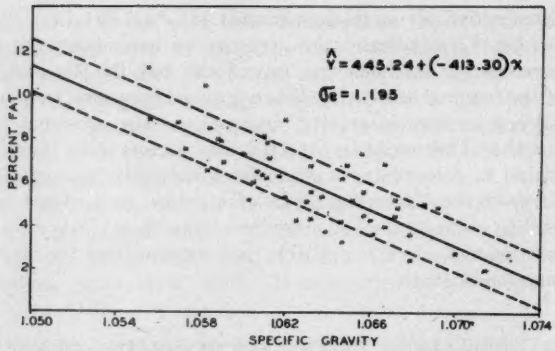

Figure 1. Regression of percent fat on specific gravity.

of 1.20%. Each increase of 0.001 in specific gravity resulted in a decrease of 0.41 in percent fat.

Figure 2 presents the relationship between specific gravity and percent water. The estimate of the regression equation was $Y=(-273.66)+325.28\,X$, while the standard error of the estimate was 1.21%. For every change of 0.001 in specific gravity, there was a change of 0.33 in percent water.

The relationship between specific gravity and percent protein is depicted in figure 3. The regression equation was $Y=(-65.38)+81.61\,X$, and the standard error of the estimate was 0.46% protein. For every change of 0.001 in specific gravity, there was a change of 0.082 in percent protein.

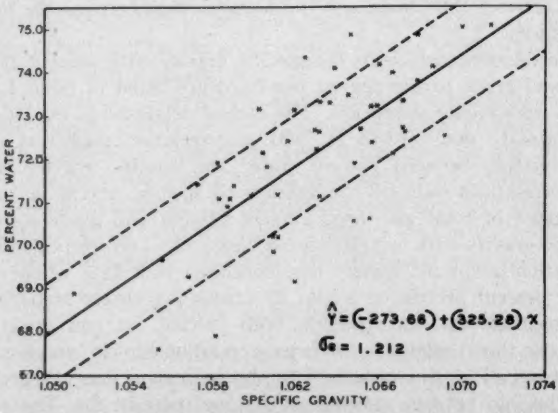

Figure 2. Regression of percent water on specific gravity.

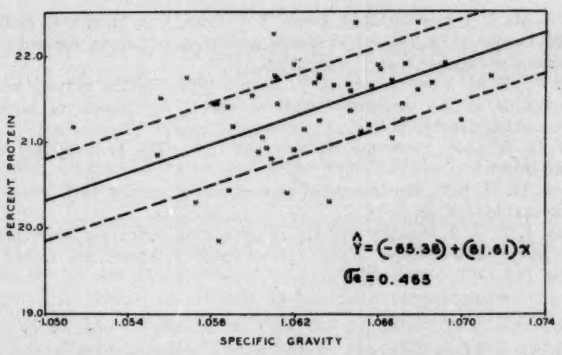

Figure 3. Regression of percent protein on specific gravity.

Summary

Specific gravity of the *Longissimus dorsi* muscle of the 9–10–11 rib section was determined in order to test the usefulness of specific gravity as an objective measure of marbling. Ether extract was used as the standard by which the validity of this measurement was determined. Correlation coefficients between specific gravity and percent fat, water, protein and grade to the nearest one-third were —.81, 0.74, 0.68 and —.68, respectively. All were highly significant.

The regression of percent fat in the rib eye on specific gravity was found to be —413.30, with a standard error of the estimate of 1.20% fat. Thus, for each increase of 0.001 units in specific gravity there was a decrease of 0.41 in percent fat. The high relationship between specific gravity and fat indicates the usefulness of specific gravity as an objective measure of marbling. Furthermore, specific gravity offers the added advantages of ease of determination and of maintainence of the meat in a saleable condition.

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THE EFFECT OF SUPPLEMENTAL VITAMIN D IN RATIONS FOR PIGS FED IN THE ABSENCE OF DIRECT SUNLIGHT [1]

RICHARD C. WAHLSTROM AND DARWIN E. STOLTE [2]

South Dakota Agricultural Experiment Station, Brookings

IT is well recognized that vitamin D is an essential nutrient for proper growth and skeletal development of swine. When swine are confined indoors or do not receive sufficient exposure to direct sunlight some supplementary feed source of vitamin D has been recommended.

Bethke *et al.* (1946) indicated that the minimum practical vitamin D requirement of growing pigs fed a ration containing 0.6% calcium and 0.45% phosphorus is approximately 90 U.S.P. units per lb. of ration.

These studies were conducted to obtain further information on the influence of supplemental vitamin D in more highly fortified rations fed to pigs in the absence of sunlight. The effects of calcium to phosphorus ratios, antibiotics and method of feeding calcium and phosphorus supplements on the need for supplemental vitamin D were also investigated.

Procedure

Experiment 1. Twenty-four weanling purebred Duroc, Poland China and Spotted Poland China pigs averaging approximately 33 lb. were allotted to six lots of four pigs each. Four of the lots were confined to indoor pens while two lots (5 and 6) had access to an outside runway.

The composition of the basal rations for all three experiments is shown in table 1. Supplements to the basal rations are shown in table 2. Vitamin D was added at a level of 90 U.S.P. units per lb. of ration in the form of irradiated yeast. Chlortetracycline was used at a level of 10 gm. per ton of ration. All rations were self-fed.

As the fastest gaining animal in each lot reached market weight it was removed and slaughtered. The femurs were removed, and length, weight, breaking strength and calcium and phosphorus content were determined. Breaking strength was determined on a section of the femur with a Tinius-Olsen dynamometer as described by Stolte (1957). Calcium and phosphorus determinations were made on the ashed samples according to A.O.A.C. methods (1950).

Experiment 2. This experiment was designed to study the effect of vitamin D supplementation in rations with calcium to phosphorus ratios of 1.5, 2.0 and 2.5.

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TABLE 1. PERCENTAGE COMPOSITION OF BASAL RATIONS

Ingredients	Experiments 1 and 2	Experiment 3	
		Ration A	Ration B
Ground yellow corn	83.1	80.7	82.8
Soybean oil meal	10.4	17.0	17.0
Tankage (60%)	5.2	—	—
Ground limestone	0.8	0.7	[a]
Steamed bonemeal	—	1.0	[a]
Trace mineral salt	0.5	0.5	[a]
Vitamin mixture [b]	0.1	0.1	0.1
Chlortetracycline	0.05 [c]	0.05	0.05

[a] A mineral mixture composed of equal parts of these three ingredients was fed free choice.
[b] Additions to each lb. of ration were as follows: riboflavin, 1 mg.; niacin 4.5 mg.; calcium pantothenate 2 mg.; choline 5 mg.; and vitamin B_{12} 4.5 mcg.
[c] Added to rations fed Lots 3 and 4 in Experiment 1 and to all lots in Experiment 2.

Two replicates each of 24 fall-farrowed pigs were used in this experiment. They were of Duroc, Hampshire and Spotted Poland China breeding and averaged 36.5 lb. The pigs were assigned to the six treatments at random from outcome groups formed on the basis of ancestry and body weights.

The same basal ration was used as in Experiment 1 except that chlortetracycline was included at a level of 10 gm. per ton and zinc carbonate at a level of 80 gm. per ton. Ground limestone was used to widen the calcium to phosphorus ratio. By chemical analysis the ratios of calcium to phosphorus were 1.8, 2.3 and 2.5 for the three rations. Irradiated yeast was added to supply 90 U.S.P. units of vitamin D per lb. of ration to the vitamin D supplemented lots.

TABLE 2. THE EFFECT OF VITAMIN D, SUNLIGHT AND ANTIBIOTICS ON GROWING PIGS (4 PIGS PER GROUP). EXPERIMENT 1

Item	Lot 1 Control	Lot 2 Vitamin D	Lot 3 Antibiotic	Lot 4 Antibiotic + vitamin D	Lot 5 Sunlight	Lot 6 Sunlight + vitamin D
Av. initial wt., lb.	33.2	33.0	33.0	33.0	31.7	33.2
Av. final wt., lb.	209.2	203.5	213.8	215.2	198.0	192.8
Av. no. days on exp.	99	95	95	95	94	103
Av. daily gain, lb.	1.79	1.80	1.90	1.92	1.77	1.56
Av. daily feed, lb.	5.70	6.02	5.62	6.34	5.74	5.05
Feed/cwt. gain, lb.	321	335	295	331	334	328
Physical measurements and chemical analyses of femurs [a]						
Length, cm.	18.10	17.38	17.92	16.95	18.00	18.60
Weight, gm.	91.08	89.10	103.50	87.89	92.08	102.20
Breaking strength (1000 lb./sq. in.)	6.73	7.13	7.74	5.66	10.33	12.71
Ash, %	64.28	65.41	64.90	66.14	65.68	64.06
Calcium, %	29.54	26.34	27.81	27.91	25.64	24.75
Phosphorus, %	12.82	11.75	12.95	13.40	10.92	13.36

[a] These figures are averages of two femurs from one pig per group.

Blood samples of from 5 to 10 ml. were taken from the tails of all pigs at the end of Experiment 2. Calcium was determined by the method of Clark and Collip (1925) and inorganic phosphorus was determined by the method of Fiske and Subbarow (1925). Analysis of the femurs was carried out as explained in Experiment 1.

Experiment 3. Eight pigs from each of the Duroc, Hampshire, Poland China and Spotted Poland China breeds were allotted into outcome groups of four pigs each on the basis of body weight and litter. The outcome groups were assigned at random to the four replicated treatments.

A corn-soybean meal basal ration was used in this experiment. The composition is shown in table 1. Lots 1 and 2 received a complete mixed ration and Lots 3 and 4 received their mineral supplement free choice. Vitamin D was added to the rations fed Lots 2 and 4 at a level of 90 U.S.P. units per lb. of ration. Each lot was confined to an indoor concrete floored pen, 8 ft. by 10 ft.

The pigs used in these experiments all had access to sunlight from 5 to 7 weeks prior to weaning and therefore had opportunity to store vitamin D. In all experiments the growth data were treated statistically by analysis of variance as outlined by Snedecor (1946).

Results and Discussion

Experiment 1. The results of the first experiment are summarized in table 2. There was no statistical difference in rate of gain between the various treatments. All groups made excellent gains with the exception of the pigs having access to sunlight and receiving supplemental vitamin D in their ration. It would appear that the slower gains of this group was due to chance and not to the treatment imposed.

The results of this experiment indicate that there is no need, as measured by growth response, for supplemental vitamin D in rations for growing pigs confined in the absence of sunlight. This is in agreement with the work of Hart and Steenbock (1922), Dunlop (1935), and Braude *et al.* (1943) but disagrees with the reports of Johnson and Palmer (1938) and Bethke *et al.* (1946). It is realized that these contradictory findings could be due to a variable storage of vitamin D in the pigs before they were put on experiment. The pigs which received the antibiotic supplemented rations gained approximately 6% faster than did their corresponding control groups. Because a deficiency of vitamin D did not exist, it was impossible to determine if the antibiotic exerted a sparing action on the supplemental need for this vitamin as reported by Lindblad *et al.* (1953) and Ross and Yacowitz (1953) in the case of the chick. The pigs fed in the absence of sunlight showed an increase in feed consumption when the ration was supplemented with vitamin D in the form of irradiated yeast. These results correspond to the findings of Johnson and Palmer

(1938), who demonstrated that the addition of irradiated yeast to the ration caused an improvement in appetite.

The physical measurements and chemical analyses of the femurs are also summarized in table 2. The length and dry fat-free weight of the femurs appear to vary only slightly, with no trend between treatments. There was a definite difference in breaking strength between the femurs of the pigs subjected to sunlight and those of the confined pigs. It is possible that the additional vitamin D obtained through the action of ultraviolet light on the provitamin in the skin may have caused this increased

TABLE 3. INFLUENCE OF VITAMIN D SUPPLEMENTATION TO RATIONS VARYING IN CALCIUM CONTENT AND FED TO PIGS CONFINED INDOORS

Treatment	No vitamin D			Plus vitamin D		
Ca-P ratio	1.8–1.0	2.3–1.0	2.5–1.0	1.8–1.0	2.3–1.0	2.5–1.0
Number of pigs[a]	6	6	6	6	6	6
Av. initial wt., lb.	37.0	37.7	38.5	38.5	38.3	37.7
Av. final wt., lb.	204.0	205.2	198.3	201.8	205.7	203.5
Av. no. days in exp.	92	90	90	89	92	93
Av. daily gain, lb.	1.83	1.86	1.80	1.84	1.84	1.80
Av. daily feed, lb.	6.10	6.35	6.31	6.38	6.34	6.34
Feed/cwt. gain, lb.	336	341	357	344	347	357

[a] Two replicated lots of 4 pigs each were allotted. One outcome group from each replicate was removed from test because of death of several pigs and poor growth of other pigs from these outcome groups.

bone strength. An increased level of vitamin D in the ration of the confined pigs might have caused this same effect. However, we did not consider a true vitamin D deficiency existed in the confined pigs because of the lowered breaking strength of the femurs since these pigs did not show any of the typical bone changes associated with vitamin D deficiency such as beading of the ribs, bow-legs or enlargement of the ends of the long bones. The bone ash percentages in all treatments were well above the levels set by Sinclair (1929) of 58 to 59% as indicative of satisfactory utilization and acceptable bone strength. It appeared that there was no rachitic effect on the bone development due to any of the treatments.

Experiment 2. The effect of varying the level of calcium in the ration, with and without vitamin D supplementation, is given in table 3. The average rate of gain was very uniform between treatments, ranging from 1.80 to 1.86 lb. per day. Increasing the level of calcium in the ration from 0.72% to 0.93%, which gave calcium to phosphorus ratios of from 1.8–1.0 to 2.5–1.0, had no effect on the rate of gain of confined pigs. Also vitamin D supplementation had no influence on the rate of gain, regardless of the calcium to phosphorus ratios used in this experiment.

The levels of blood calcium and inorganic phosphorus shown in table 4 did not vary significantly between ration treatments. With the exception of the low phosphorus level of the group receiving high calcium and no

TABLE 4. AVERAGE CHEMICAL ANALYSES OF THE BLOOD AND CHEMICAL ANALYSES AND PHYSICAL MEASUREMENTS OF THE FEMURS OF PIGS FROM EXPERIMENT 2

Treatment	No vitamin D			Plus vitamin D		
Ca-P ratio	1.8–1.0	2.3–1.0	2.5–1.0	1.8–1.0	2.3–1.0	2.5–1.0
Mg./100ml. of blood serum						
Calcium	11.79	13.00	11.98	14.43	11.74	12.78
Phosphorus	9.42	8.81	6.85	9.15	8.40	9.34
Femur analysis						
Ash, %	71.96	72.41	71.24	71.85	72.63	72.18
Calcium, %	32.01	29.76	29.55	33.43	32.56	32.99
Phosphorus, %	14.21	15.15	14.34	14.85	15.12	14.94
Length, cm.	17.00	17.60	17.75	17.45	17.30	17.70
Weight, gm.	104.10	109.98	104.45	107.70	110.02	113.50
Breaking strength (1000 lb./sq. in.)	11.88	9.17	9.76	12.20	10.67	11.62

vitamin D, the calcium and phosphorus levels presented are in line with the normal levels stated by Hughes (1936) of 11.93 mg. calcium and 8.34 mg. phosphorus per 100 ml. of blood. In all treatments the relation of the level of serum calcium to the absence of rickets is in agreement with the results of Dunlop (1935). He found that symptoms of rickets did not appear until the blood calcium dropped below 8 mg. per 100 ml. of blood. The bone analyses indicated little difference between treatments. There appeared to be a trend toward greater calcium deposition in the bone when vitamin D was present in the ration.

Experiment 3. Summaries of the gain and feed efficiency data for this experiment are presented in table 5. An analysis of variance of the daily

TABLE 5. THE EFFECT OF VITAMIN D SUPPLEMENTATION IN SWINE RATIONS SUPPLEMENTED WITH MINERALS OR WITH MINERAL SUPPLEMENT FED FREE-CHOICE EXPERIMENT 3 [a]

Treatment	Group	Av. initial wt.	Av. final wt.	Av. daily gain [b]	Av. daily feed	Feed per 100 lb. gain
		lb.	lb.	lb.	lb.	lb.
Ration A	I	32.3	204	1.78	5.33	298
	II	37.3	201	1.80	5.42	302
	Av.	34.8	202	1.79	5.38	300
Ration A+Vit. D	I	32.5	203	1.81	5.57	308
	II	37.0	197	1.80	5.59	311
	Av.	34.8	200	1.80	5.58	310
Ration B	I	32.0	136	1.06	3.97	374
	II	37.5	129	1.03	4.11	399
	Av.	34.8	132	1.05	4.04	385
Ration B+Vit. D	I	32.2	158	1.29	4.05	314
	II	37.0	190	1.56	5.35	344
	Av.	34.6	174	1.42	4.71	330

[a] Four pigs per group.
[b] L.S.D. at $P<0.05=0.29$; and at $P<0.01=0.39$.

gains showed that the treatments exhibited a statistically highly significant effect ($P<0.01$). This difference was shown to be due largely to the different methods of feeding.

The group of pigs fed ration A, the complete mixed ration, made rapid and efficient gains regardless of vitamin D supplementation. This ration, by calculation, contained 0.61% calcium and 0.48% phosphorus. These results are in agreement with Experiments 1 and 2 in which there was no need for supplemental vitamin D when complete mixed rations were fed to growing pigs in the absence of sunlight. Bethke *et al.* (1946) reported a fundamental need for supplemental vitamin D when rations containing approximately 0.60% calcium and 0.45% phosphorus were fed to growing pigs. The reasons for the difference in the results reported here and those of Bethke *et al.* are not obvious. However, there were some differences in experimental procedure that are noteworthy. The latter authors used a four-week depletion period which was not used in the experiments reported herein. They also fed a basal ration that was not supplemented with B vitamins and antibiotic as was the basal ration used in these trials. The rate of gain obtained in these trials was much faster than that obtained in the Ohio trials and as a result the deficient rations were fed for a shorter period of time.

Feeding ration B, a corn-soybean meal ration with a mineral supplement fed free-choice, resulted in very poor growth and rickets in five of the eight pigs. Symptoms were lameness, crooked legs, poor growth and soreness of feet and legs which was evidenced by the objection of these pigs to stand or walk. One pig developed posterior paralysis after 9 weeks on the experiment and was sacrificed. Adding vitamin D to this ration resulted in increased rate of gain but did not give complete protection against rickets. Two of the eight pigs fed this supplemented ration developed rickets and although the other six pigs did not show visible symptoms of rickets they did not gain as rapidly as did the pigs fed ration A. Approximately 2 to 2.5 lb. of mineral supplement were consumed per pig during the experimental period. It is quite evident that this did not provide adequate calcium for these pigs. On the basis of the free-choice consumption of ration B and the mineral supplement, these pigs were consuming a ration containing approximately 0.20% calcium and 0.37% phosphorus. This is considerably below the National Research Councils' (1953) recommendation for calcium for growing pigs. It appears that adding vitamin D to ration B did enhance the availability or utilization of the calcium as the pigs grew faster and more efficiently and fewer animals developed rickets than when vitamin D supplementation was absent.

Summary

Three experiments were conducted to study the effects of vitamin D supplementation of rations for growing swine raised in the absence of

direct sunlight. The addition of 90 U.S.P. units of vitamin D per lb. to mixed rations complete in other known dietary factors resulted in little difference in rate of gain, calcium and inorganic phosphorus content of the blood or calcium, phosphorus and total ash content of femurs.

A significant reduction in growth rate occurred when a low calcium ration was fed along with a mineral supplement free-choice. Five of eight pigs fed this ration also developed symptoms of rickets. Supplementing this ration with vitamin D increased gains and decreased the number of pigs with visible symptoms of rickets. It was observed that the pigs fed in this manner did not consume enough calcium to meet the requirements for this nutrient.

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THE INFLUENCE OF AGE, SEX, AND SEASON OF BIRTH OF CALF, AND AGE OF DAM ON PREWEANING GROWTH RATE AND TYPE SCORE OF BEEF CALVES

THOMAS J. MARLOWE AND JAMES A. GAINES

Virginia Agricultural Experiment Station, Blacksburg

AN attempt has been made to evaluate the influence of age, sex, and season of birth of calf, and age of dam on the preweaning growth rate and type score of Virginia beef calves. If these sources of variations can be accurately evaluated, such knowledge will enable the breeder to measure the genetic difference between calves with a higher degree of accuracy. Because all of the data for this study were not available at one time, two separate analyses were done, one on creep-fed calves and one on non-creep-fed calves.

A recent publication by Koch and Clark (1955) contains a review of the literature pertinent to this study, and only relevant publications are cited in the present discussion.

Data. The data in this study were the records of 4,166 non-creep-fed and 2007 creep-fed calves in the Virginia performance testing program over a four-year period, 1953–56, inclusive. Almost half of the records were obtained in 1956. The data were pooled over all years and over 44 Angus, 19 Hereford, and 3 Shorthorn herds located in the state.

All calves were weighed and scored for type when they were between 90 and 300 days old. Herds in which some of the calves were creep-fed and some were not were excluded from the study. Growth rate was determined by subtracting birth weight from preweaning weight and dividing by age of calf in days.

The type scoring system included five classifications, subdivided into thirds of grades, as follows: Fancy 15–17; Choice 12–14; Good 9–11; Medium 6–8; and Common 3–5. Type scoring was done primarily by one person, the official type scorer for the Virginia program.

The data were summarized in a table of means as shown in table 1.

Method

Estimates of the effects mentioned above were computed using conventional least squares methods. Each calf was classified in four different ways, according to its age, sex, season of birth, and age of its dam. It was assumed that there were no interactions, so the model could be written:

$$y_{ijklm}=\mu+a_i+s_j+b_k+c_l+e_{ijklm}$$

where $i=1 \ldots 7$; $j=1 \ldots 3$; $k=1 \ldots 4$; and, $l=1 \ldots 9$.

The effect of a change in position with regard to one classification was assumed to be the same, regardless of the position of the calf with respect

TABLE 1. UNADJUSTED MEANS OF PERFORMANCE TRAITS STUDIED

Effects studied	No. of calves		Av. daily gain		Type score [b]	
	Non-creep	Creep	Non-creep	Creep	Non-creep	Creep
Age of calf (days)						
90–120	201	108	1.69	1.71	10.7	11.8
121–150	607	267	1.70	1.74	11.2	11.9
151–180	932	389	1.70	1.82	11.4	11.9
181–210	1074	487	1.66	1.78	11.6	12.1
211–240	785	450	1.63	1.75	11.9	12.2
241–270	459	230	1.54	1.72	11.6	12.3
271–300	108	76	1.42	1.49	11.4	12.3
Sex of calf						
Bulls	511	443	1.79	1.89	11.7	12.1
Heifers	2115	991	1.58	1.67	11.7	12.3
Steers	1540	573	1.70	1.80	11.2	11.7
Season of birth [a]						
Dec. 16–Mar. 15	2163	1009	1.65	1.76	11.7	12.1
Mar. 16–May 31	1425	639	1.70	1.79	11.3	12.0
June 1–Aug. 31	146	172	1.54	1.72	10.6	12.1
Sept. 1–Dec. 15	432	187	1.50	1.63	11.7	12.3
Age of dam (years)						
0.0– 2.5	311	138	1.46	1.59	10.7	11.3
2.5– 3.5	607	316	1.58	1.68	11.3	12.0
3.5– 4.5	657	368	1.63	1.76	11.4	12.0
4.5– 5.5	655	272	1.66	1.79	11.6	12.5
5.5– 6.5	522	311	1.70	1.73	11.8	12.1
6.5– 7.5	408	175	1.74	1.83	11.8	12.3
7.5– 8.5	275	122	1.68	1.88	11.7	11.8
8.5– 9.5	217	101	1.73	1.85	11.4	12.4
9.5–18	514	204	1.67	1.79	11.6	12.2
Mean	4166	2007	1.65	1.76	11.5	12.1

[a] Average birth dates were Feb. 6, Apr. 13, July 7, and October 20 for non-creep and Feb. 10, Apr. 15, July 15, and Oct. 31 for creep-fed calves, respectively.
[b] Type score code: Fancy 15–17; Choice 12–14; Good 9–11; Medium 6–8.

to the other classifications. Assuming further that the e_{ijklm}'s had an expectation of zero, had the same variance σ^2, and were uncorrelated, least squares could be used.

In the model y_{ijklm} is a preweaning weight or a type score on a calf, μ is the general mean of all calves; a_i is an effect due to the age of the calf; s_j is an effect due to the sex of the calf; b_k is an effect due to the season in which the calf was born; c_l is in effect due to the age of the cow; and, e_{ijklm} is a random effect peculiar to the calf.

Normal equations were set up according to the model, the resulting matrices being 20x20. Inversion of the matrices and estimation of the parameters was accomplished as outlined by McGilliard (1955).

Results and Discussion

Lease Squares Estimates of Effects. Solution of the normal equations yielded the estimates shown in table 2. These estimates would serve as adjustment factors if the signs were reversed.

TABLE 2. LEAST SQUARES ESTIMATES OF THE INFLUENCE OF AGE, SEX, AND SEASON OF BIRTH OF CALF, AND AGE OF DAM ON PRE-WEANING GROWTH RATE AND TYPE SCORE

	No. of calves		Av. daily gain				Type-score[a]			
Effects studied	Non-creep	Creep	Non-creep		Creep		Non-creep		Creep	
Age of calf (days)										
90–120	201	108	.03	.02[b]	−.08	.03[b]	−.8	.13[b]	−.4	.19[b]
121–150	607	267	.02	.01	−.05	.02	−.4	.10	−.3	.14
151–180	932	389	.01	.01	.03	.02	−.2	.08	−.3	.12
181–210	1074	487	00		00		0		0	
211–240	785	450	−.02	.01	−.03	.02	.2	.09	.1	.11
241–270	459	230	−.06	.02	−.08	.02	.0	.11	.1	.13
271–300	108	76	−.17	.03	−.23	.04	−.1	.19	.0	.22
Sex of calf										
Bulls	511	443	.07	.01	.10	.02	.6	.09	.4	.10
Heifers	2115	991	−.12	.01	−.13	.02	.5	.06	.5	.09
Steers	1540	573	00		00		0		0	
Season of birth										
Dec. 16–Mar. 15	2163	1009	00		00		0		0	
Mar. 16–May 31	1425	639	.04	.01	.03	.02	−.1	.08	.2	.11
June 1–Aug. 31	146	172	−.09	.02	−.04	.02	−.9	.16	.2	.14
Sept. 1–Dec. 15	432	187	−.12	.02	−.03	.03	−.1	.11	.2	.15
Age of cow (years)										
0.0–2.5	311	138	−.30	.02	−.25	.03	−.9	.13	−1.0	.19
2.5–3.5	607	316	−.17	.02	−.17	.03	−.4	.11	−.3	.16
3.5–4.5	657	368	−.11	.02	−.07	.03	−.3	.11	−.3	.15
4.5–5.5	655	272	−.08	.02	−.05	.03	−.1	.11	.1	.16
5.5–6.5	522	311	−.04	.02	−.09	.03	.1	.11	−.3	.16
6.5–7.5	408	175	00		00		0		0	
7.5–8.5	274	122	−.06	.02	.04	.03	−.1	.14	−.5	.19
8.5–9.5	217	101	−.01	.02	.03	.04	−.3	.15	−.1	.21
9.5–18	514	204	−.07	.02	−.04	.03	−.3	.12	−.2	.17
Mean	4166	2007	1.81		1.90		11.6		12.0	

[a] Type score code: Fancy 15–17; Choice 12–14; Good 9–11; Medium 6–8.
[b] Values are ±.

Age of Calf. The age of the calf was arbitrarily divided into seven equal periods of 30 days in length (90 to 300 days). No significant differences were apparent in the growth rates of the non-creep-fed calves from 90 to 210 days of age; a slight decline was evidenced in the next period and a rather sharp decline from 241 to 300 days of age. The decline in the older age groups was thought to be due largely to three factors: (1) The cows were approaching the end of the lactation period, (2) the growth period extended farther into the period of poorer grazing conditions, and (3) some of the older calves were weaned prior to the time they were weighed and graded.

The influence of age of calf was greater for the creep-fed group. Both the younger and older age groups gained less rapidly than the creep-fed calves which were from 150 to 240 days of age. Perhaps the younger calves did not consume enough of the creep ration to influence their growth, and the older calves were influenced by the same factors listed above for non-creep-fed calves.

The estimates of influence of age of calf on type score were of statistical significance at the early ages in the non-creep-fed calves, but none of the estimates for either group were felt to be of practical significance, since they represented such small fractions of a grade.

These findings are in general agreement with those found in the literature with respect to growth rate (Koch and Clark, 1955; Rollins *et al.*, 1952; Hoover *et al.*, 1956). There appears to be very little information in the literature on the influence of age of calf on its type rating. Koch and Clark (1955) found a small positive relationship between age at weaning and weaning score.

Sex of Calf. The sex of the calf influenced growth rate significantly in both feeding treatments, but had little influence on type score. Bull calves grew approximately 5% faster than steer calves and steer calves grew approximately 8% faster than heifer calves. The differences were slightly larger in the creep-fed groups. Adjusted to a weaning age of 210 days, the bulls were 16 lb. heavier than steers and the steers 30 lb. heavier than heifers. These findings are in general agreement with those of other workers who have found males to be 25 to 68 lb. heavier than females at 210 days of age and little or no difference in type scores due to sex.

Season of Birth. Approximately 75% of the calves in this study were purebred. Calves are dropped in most purebred herds in all seasons of the year. Therefore, the year was arbitrarily divided into four seasons, as follows: (1) Dec. 16–Mar. 15, (2) Mar. 16–May 31, (3) June 1–Aug. 31, and (4) Sept. 1–Dec. 15. Season of birth of calf appears to be of statistical and practical significance for growth rate of non-creep-fed calves, but of little practical importance on growth rate of creep-fed calves or type score in either group. Non-creep-fed calves born during June through December grew about 0.1 lb. per day slower than those born during February through May.

The seasonal influence on type score was important only for the non-creep-fed calves born between June 1 and September 1. Calves born in that period scored approximately one-third of a grade lower than calves born in the other seasons. Creep-feeding appeared to have an important influence in equalizing type scores over all seasons.

Age of Dam. The method used in this study was to compare averages of all records made at each age of dam. As explained by Lush and Shrode (1950), this would bias the estimates upward from the true age effect because some of the cows were culled at each age, which means that

each succeeding age contained a larger share of high producing cows than at the preceding age. However, a study by Koch and Clark (1955) indicated that the magnitude of the bias is small.

The most important source of variation in growth rate and type score found in this study was attributed to differences in age of the dams. The estimates of this effect indicate that maximum production was obtained from the 6- to 10-year-old cows. The largest difference was between the 2- and 3-year-old cows. The magnitude of the differences decreased with each successive age group until maximum production was reached. The continued high level of performance of the older cows could probably be attributed to the fact that a considerable amount of selection had been practiced in these herds, and most of the poorer producers had been removed prior to reaching the older ages.

These findings agree fairly well with those reported in the literature by other workers. Koch and Clark (1955), Burgess *et al.* (1954), Rollins and Guilbert (1954), and Koger and Knox (1954) have reported maximum production from cows within the 6- to 10-year-old age range. Gifford (1953) reported that milk production in Hereford cows was greatest at 6 years of age. Kock and Clark recommended adding 0.23, 0.10, and 0.13 lb. per day to the growth rate of calves out of 3-, 4-, and 10-year-old cows, respectively. Rollins and Guilbert found that cows in the optimum age range produced calves that grew 0.17, 0.10, and 0.15 lb. per day faster than calves out of 3-, 4-, and 12 to 14-year-old cows, respectively, and weighed 21, 13, and 18 lb. more at 240 days of age.

Creep-feeding. Creep-feeding of calves appeared to increase their growth rate approximately 0.1 lb. per day and their type score approximately 1/7 of a grade. These differences are only rough approximations, however, because the study was not designed to measure this effect and other variables were included in the estimate. The most striking influence that creep-feeding exerted on the effects studied was to decrease the influence of season of birth. It stimulated growth in summer and fall calves and was quite effective in equalizing type score over all seasons.

Application of Estimates to 1957 Virginia BCIA Calf Records. It was the opinion of the authors that adjustment of performance data should be restricted to the major sources of variation. More risk is involved in over adjusting than in under adjusting. Before replacing a producing cow with a younger one the breeder should be reasonly certain that the production potential of the younger animal is at least as good as the one being replaced. If the adjustment tended to over correct, selection, in which younger cows replaced older ones, might lead to disappointment. Therefore, corrections should be made only when the influence is real and large enough to overbalance random errors in measurement.

In order to check the reliability of the estimates obtained from the 1953–56 data, they were used to adjust the average daily gains of 3147 non-creep-fed calves weighed in 1957. Since most calves were weighed be-

tween 120 and 240 days of age and since the differences due to age of calf were small, no correction was applied for age of calf.

All calves were adjusted to a steer basis for convenience in making comparison. Adjusting to a steer basis involved less adjustment of the actual records than would have been required to adjust the records to the performance level of bulls.

Examination of the average daily gains by months indicated that a more logical division of seasons would be to pool all calves into two groups, January 1 through May 31, and June 1 through December 31. An adjustment was applied to the second group.

TABLE 3. COMBINED ADJUSTMENTS APPLIED TO 1957 VIRGINIA BCIA CALF RECORDS

Sex of calf	Bull		Heifer		Steer	
Season of birth	1	2	1	2	1	2
Age of cow, years						
2	1.15	1.22	1.27	1.36	1.20	1.27
3	1.06	1.12	1.17	1.25	1.10	1.17
4	1.02	1.08	1.13	1.20	1.06	1.12
5	1.00	1.06	1.11	1.18	1.05	1.10
6–10	0.96	1.02	1.07	1.13	1.00	1.06
11–13	1.00	1.07	1.12	1.19	1.05	1.11
14 plus	1.10	1.17	1.22	1.30	1.15	1.22

The performance of calves from dams which were six to 10 years of age were not adjusted. Corrections were applied to the gains of calves from dams which were 2, 3, 4, 5, and over 10 years of age. Since all cows 10 years old and older were grouped together in the study, the adjustments for cows over 10 years of age shown in table 3 were based on the unadjusted means of these groups.

Multiplicative factors were used because of the ease of making IBM calculations. These are shown in table 3.

If the records from which the estimates were obtained were truly representative of beef calves in Virginia, one would expect the 1957 sub-group means to be approximately the same within each classification. Results of these adjustments are shown in table 4, along with the number of calves in each class.

In general, the adjustment factors, when applied to the 1957 calf records, did a satisfactory job of equalizing the sub-class means. The conclusion was substantiated that no correction for age of calf effect was necessary. The 6% correction applied to adjust all calves to the January 1 through May 31 season of calving was not large enough to equalize these groups.

No adjustment was made for any of the effects studied on type score because the differences appeared to be too small to be of any practical

importance. Calves are scored to the nearest grade point (one third of a standard grade), and in no case would a correction for type score have resulted in a change as great as one grade point.

Summary

The purpose of this study was to evaluate the influence of age, sex, and season of birth of calf, and age of dam on the preweaning growth rate and type score of Virginia beef calves.

TABLE 4. ADJUSTED MEANS OF 1957 CALF RECORDS

Age of calf (days)[a]	No. of calves	Mean	Age of cow	No. of calves	Mean
90–120	164	1.78	2	226	1.75
121–150	464	1.78	3	393	1.72
151–180	575	1.79	4	489	1.71
181–210	802	1.75	5	380	1.75
211–240	686	1.67	6	420	1.74
241–270	321	1.70	7	264	1.71
271–300	135	1.62	8	250	1.73
Total	2943	1.74	9	162	1.73
			10 Plus	359	1.77
			Total	3147	1.74
Sex of calf			Season of birth		
Bulls	242	1.76	Jan. 1–May 31	2460	1.75
Heifers	1512	1.73	June 1–Dec. 31	687	1.69
Steers	1393	1.74	Total	3147	1.74
Total	3147	1.74			

[a] No adjustment for age of calf.

The study included 6,173 calves, divided into two groups for analysis. One group included 4,166 non-creep-fed calves and the other included 2,007 creep-fed calves. The data were pooled over a four-year period (1953–1956, inclusive) and over 44 Angus, 19 Hereford, and 3 Shorthorn herds.

The data were arranged into sets of normal equations, and least squares estimates of the various effects were computed.

No significant differences were apparent in the growth rate of non-creep-fed calves from 90 to 210 days of age. There was a slight decline from 211 to 240 days and a rather sharp decline after 240 days. Creep-fed-calves under 150 and over 240 days of age appeared to be at a greater disadvantage than non-creep-fed calves in those same age groups. Type scores were not influenced sufficiently by age of calf to be of any practical importance.

Sex of calf influenced growth rate significantly in both the creep-fed and non-creep-fed groups but had little effect on type score. Bull calves

grew approximately 5% faster than steer calves, and steer calves grew approximately 8% faster than heifer calves.

Season of birth had a significant influence on growth of non-creep-feed calves, but was of no practical importance on growth of creep-fed calves or type score in either group.

Age of dam was the most important source of variation studied with the largest differences occurring among the younger age groups. Maximum production was obtained from cows in the six- to ten-year-old age group.

The reliability of the estimates was checked by applying them to the performance records of 3,147 non-creep-fed calves weighed in 1957. They were found to be satisfactory for equalizing sub-class means.

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MICROFLORA OF INTESTINAL CONTENTS AND FECES OF PIGS FED DIFFERENT DIETS INCLUDING PIGS SHOWING PARAKERATOSIS

D. J. Horvath,[1] H. W. Seeley, R. G. Warner and J. K. Loosli

Cornell University, Ithaca, New York

ENUMERATIONS of the fecal microflora of the pig have been conducted by several groups of workers in conjunction with studies of the effects of antibiotics, e.g., Larson and Hill (1955). Willingale and Briggs (1955) studied the flora of the lower tract of the pig and concluded that it was essentially the same as that of the feces, if corrections for differences in dry matter were imposed. However, little is known of the flora of the upper tract, especially the stomach, and no report takes into account the initial numbers of organisms entering the tract with the feedstuffs. Consequently, the collection of such data seemed desirable.

Moreover, because of information derived from the study of the approximate contributions of various groups of bacteria in the tract of man and the rat and also in some carnivores and herbivores, there would be some value in estimating the number of organisms in the digestive tract. This is a logical background for other related studies leading towards more direct answers to the question, What is the net contribution of the flora to the "ultimate diet" of the pig?

Experimental

Four individually-penned, hand-fed, growing-finishing pigs were studied in each of two experiments in the summers of 1955 and 1956. The four diets used were intended to contain approximately 10% more fat, fiber, or protein, respectively, than did the corn-soybean oil meal basal diet (table 1).

After at least one week on experiment, fresh fecal samples were collected weekly from each pig for 5 to 6 weeks. Samples were brought to the laboratory in sterile containers and a portion was dried at 95° C. A second portion of 1 to 2 gm. was used as inoculum. This was suspended in 100 ml. of sterile 0.5% saline and triplicate serial dilutions of selective media prepared. Numbers estimated were calculated to a dry matter basis. On one occasion samples were obtained from the same pig at 7 a.m. and again at 4 p.m. to permit estimation of within-day variation.

Media used were as follows: Difco MacConkey Broth incubated at 37° C. for 48 hours for coliforms; Difco S.F. Broth incubated at 45° C. for 48 hours for enterococci; Difco S.L. Agar in double pour plates incu-

[1] Present address: Department of Animal Husbandry, University of West Virginia, Morgantown.

bated at 37° C. for 5 days for lactobacilli; Difco Malt Agar adjusted to pH 3.5, incubated at 37° C. for 48 hours for molds and for 5 days for yeasts. In addition the Proteus medium of Zarett and Doetsch (1949) and Thompson's (1939) modified Wilson-Blair medium for H_2S-producing clostridia were also employed occasionally. Broth counts were based on the most probable numbers system, whereas agar counts were made directly.

In the second phase of the study, as each pig reached 200 lb. in body weight, it was slaughtered according to routine procedures of hanging,

TABLE 1. COMPOSITION OF DIETS USED IN THE STUDY OF INTESTINAL MICROFLORA

Ingredient	Basal	High fat	High fiber	High protein
	%	%	%	%
Corn	84	74	66	64
Soybean oil meal	9	9	2	9
Ground alfalfa hay	5	5	30	5
"Brown grease"	—	10	—	—
Meat scraps	—	—	—	20
Iodized salt	1	1	1	1
$CaCO_3$	0.5	0.5	0.5	0.5
Steamed bone meal	0.5	0.5	0.5	0.5
Fortafeed [a]	+	+	+	+
Trace minerals [b]	+	+	+	+
A.Q.V.[c]	+	+	+	+

[a] Fortafeed (Lederle). A vitamin supplement added at the level of ¼ lb. per 500 lb. ration.

[b] Trace minerals. Based on analysis of a corn-soybean oil meal diet by Noland *et al.* (1951) and the N.R.C. (1953) recommendations. The following mixture of trace minerals was added per 500 lb.: 2 gm. $CoCl_2 \cdot 6H_2O$; 12 gm. $CuSO_4 \cdot 5H_2O$; 12 gm. $FeSO_4 \cdot 7H_2O$; 20 gm. $MnCl_2 \cdot 4H_2O$; and 20 gm. $ZnSO_4 \cdot 7H_2O$. In Experiment II, 50 p.p.m. Zn (50 gm. $ZnSO_4 \cdot 7H_2O$) were used.

[c] Anthraquinone violet (A.Q.V.) was added as 12 gm. per 100 lb. in Experiment I.

bleeding, scalding, scraping, hanging and eviscerating. In each instance, the pigs were fed at 7 a.m. and bled at 9 a.m. so that the post-prandial interval was constant. The viscera were "stripped" into porcelain pans which had been washed with alcohol and flamed. Bacteriological methods were as above except that the stomach contents were always used as the first inoculum since pilot studies had shown the pH of this material to change quite rapidly, whereas the pH of the intestinal contents was essentially unchanged after one hour.

A feed sample was taken on the day of slaughter and similarly analyzed. An additional pair of pigs, X and Y was slaughtered following nembutal injections in an effort to minimize the possibilities of reverse peristalis or contamination of the stomach contents with duodenal ingesta due to hanging, scraping and eviscerating the animals. In the first instance 10 ml. of nembutal (50 mg. per ml.) were given intraperitoneally. This dose did not fully anesthetize pig X and he had to be stunned before bleeding. For pig Y, 25 ml. of nembutal were administered and the pig succumbed quietly and was bled without any thrashing. In both cases, the abdominal wall was

promptly opened and the stomach tied off, removed and sampled. The pigs were not lifted out of the horizontal plane. Bacteriological methods were essentially the same as described above except that the 45° C. water bath was inoperative and the tubes of S.F. medium for pig X were lost and the S.F. tubes for pig Y were therefore incubated at 37° C. Gram stains were prepared to ascertain if the medium still supported only gram (+) cocci at this temperature. Since the pigs were transported to the kill-room pen the previous day, the only restraint used was for the brief interval necessary for intraperitoneal injection.

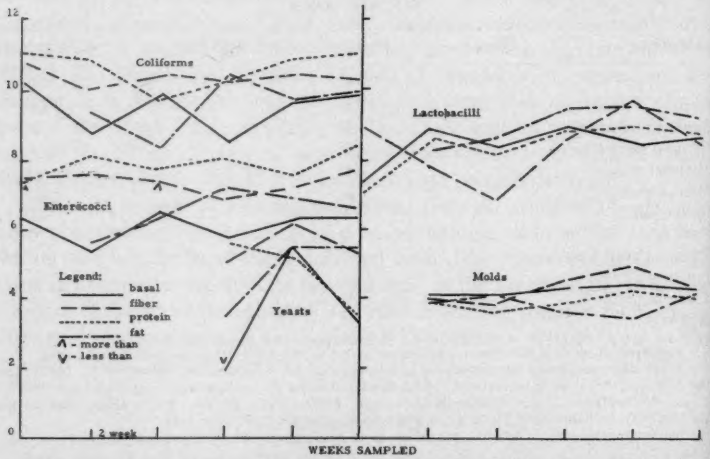

Figure 1. Fecal counts of bacteria, yeasts and molds of pigs on different diets. Replicate 1. The terms "more than" or "less than" indicate the maximum or minimum possible number of organisms in the sample in question when the dilution range was not properly chosen.

Results and Discussion

The counts of the various bacterial populations obtained, expressed as the log of the number of viable cells per gram dry matter, are presented graphically as figures 1 and 2.

The numerically dominant organisms, of those studied, were the coliforms. This is what Burrows (1954) implied in man, but Willingale and Briggs (1955) found only approximately 10^6 coliforms in pigs, using the same medium. In the published reports of floral antibiotic studies, the control pigs have also generally had less than 10^{10} coliforms.

The numbers of enterococci and lactobacilli, approximately 10^8, are less than those noted by Willingale and Briggs (1955). However, their method was to isolate organisms from high dilution positive tubes of total count

media. The difference, therefore, may reflect a limitation of selective media.

Numbers of yeast and molds were both approximately 10^4, but some difficulty was initially encountered in establishing yeast counts and this was the most variable series studied.

Proteus spp. were estimated to be present at approximately 10^4, but difficulty was encountered in use of the medium and it was not regularly employed.

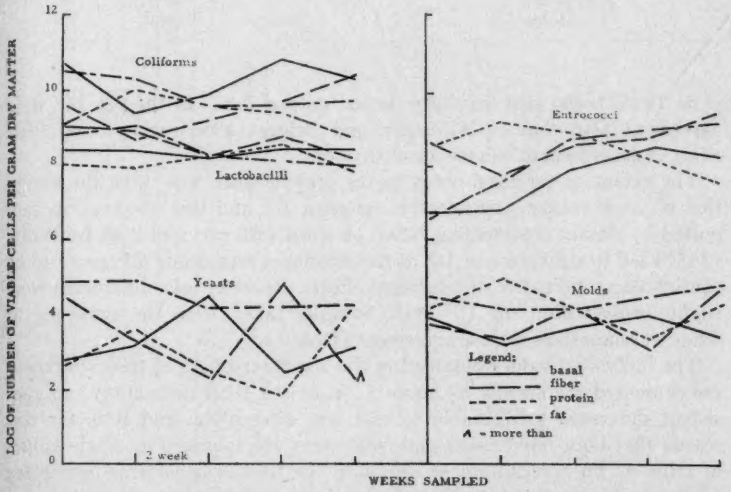

Figure 2. Fecal counts of bacteria, yeasts and molds of pigs on different diets. Replicate II. The terms "more than" or "less than" indicate the maximum or minimum possible number of organisms in the sample in question when the dilution range was not properly chosen.

Clostridium spp. were not found at any time with the medium employed, a finding which agrees with the report of Wahlstrom *et al.* (1954). On the other hand, White (1957) used Mossel *et al.* (1956) medium with plain agar caps and found dense black colonies equivalent to approximately 10^5 clostridia in tubes inoculated with feces of a pig from the Cornell University herd fed a diet similar to the basal diet used in the experiment reported here. This agrees with the findings of several workers. Baker *et al.* (1950) consider *Clostridium butyricum* to be the primary amylolytic agent in the cecum of the pig.

In the time interval studied, no marked consistent dietary effects were found. This was somewhat unexpected in light of the differences in diets in terms of practical swine feeding, and it implies a highly "buffered" condition of the intestinal tract. This concept contradicts the work of Quinn

TABLE 2. COMPARISON OF COUNTS AT TWO SAMPLING TIMES BASED ON ONE PIG ON THE HIGH PROTEIN DIET. (LOG OF THE NUMBER OF VIABLE ORGANISMS PER GRAM OF FECAL DRY MATTER)

Organisms	A.M.	P.M.
Coliforms	11.6	10.2
Enterococci	8.6	9.2
Lactobacilli	8.5	9.2
Yeasts	5.4	5.0
Molds	4.5	4.2

et al. (1953) who said that there is no "normal flora" of the pig, but is in agreement with that of Willingale and Briggs (1955) who found little effect of grass meal or fish meal substitutions.

The extent of variation noted in the present work was, with the exception of yeast counts, consistently less than 10^2 and this observation supported by similar observations based on work with rats and dogs by White (1957) led to the choice of 10^2 as the minimum repeatable difference to be considered as indicative of treatment effects. Between year differences were slight, being within the 10^2 limit. Samples taken from the same pig at different hours showed close agreement (table 2).

The individual values obtained in the slaughter phase of the experiment are presented graphically as figure 3. As in the fecal flora study, no consistent difference attributable to diet was discernible, and it is for this reason that both replications and treatments are averaged as single values in table 3. The precaution of sampling the feed is considered important when selective media are employed.

The graphic profiles suggest several things: the region of greatest microbial activity is the large intestine; the area of least activity may be,

TABLE 3. COUNTS OF THE FEED AND GASTROINTESTINAL CONTENTS OBTAINED ON THE DAY OF SLAUGHTER.[a] AVERAGE[b] LOG OF THE NUMBER OF VIABLE CELLS PER GRAM OF DRY MATTER

Site	Coliforms	Enterococci	Lactobacilli	Yeasts	Molds
Feed	5.4	4.9	3.0	2.8	3.3
Stomach	7.7	6.8	7.9	4.6	3.5
Small intestine	9.3	6.7	7.8	4.6	3.5
Large intestine	9.2	8.0	8.2	4.4	4.0
Feces[c]	9.9	7.6	8.5	4.1	4.0

[a] It was also observed that approximately 10^4 to 10^5 presumptive *Veillonella spp.* occurred per swabful of small or large intestinal contents from a freshly slaughtered pig. The medium used was that of Rogosa (1956) but the Gram (—) cocci or diplococci obtained did not appear to be as strictly anaerobic as *Veillonella spp.* are usually considered to be.

[b] Each figure is the average of values from 8 animals, except enterococci which includes only 7.

[c] Average values from data obtained prior to slaughter.

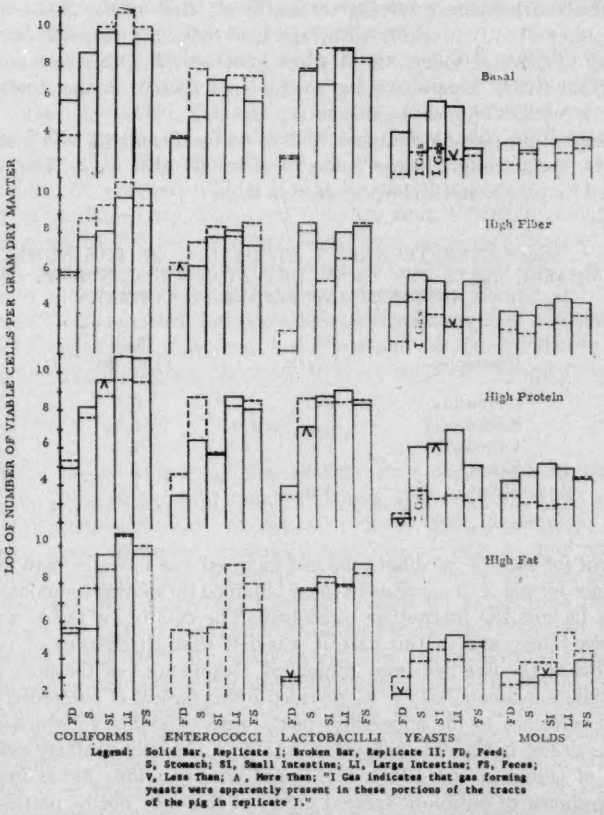
LOG OF NUMBER OF VIABLE CELLS PER GRAM DRY MATTER
Basal
High Fiber
High Protein
High Fat
I Gas
V
FD S SI LI FS
COLIFORMS
ENTEROCOCCI
LACTOBACILLI
YEASTS
MOLDS
Legend: Solid Bar, Replicate I; Broken Bar, Replicate II; FD, Feed; S, Stomach; SI, Small Intestine; LI, Large Intestine; FS, Feces; V, Less Than; Λ, More Than; "I Gas indicates that gas forming yeasts were apparently present in these portions of the tracts of the pig in replicate I."

Figure 3. Numbers of bacteria, yeasts and molds in intestinal areas of pigs at slaughter as influenced by different diets. The terms "more than" or "less than" indicate the maximum or minimum possible number of organisms in the sample in question when the dilution range was not properly chosen.

with the exception of lactobacilli, the stomach; the "plateau" profile of molds implies an inert transient form.

Despite the relatively low counts in the stomach, the stomach was not sterile. Lactobacilli, which are known to be quite acid tolerant, were consistently present in the stomach at levels more than 10^2 greater than those of the feed. Moreover, the characteristically acid-tolerant yeasts may be active in the stomach. Gas production by yeasts was noted in the malt agar plates of ingesta from 3 of 4 stomachs of pigs used in the first experiment. This observation recalls the bloating and fermentation noted in young pigs

fed refined carbohydrate (Becker *et al.*, 1954; Bell *et al.*, 1950). Porter and Rettger (1940), working with rats, also took care to point out that, contrary to classical beliefs, the stomach is not sterile. Other workers have shown that gastric urease in some species (not present in the stomach of pigs) is a product of microorganisms, not the host.

Because of the possible contamination of stomach contents with intestinal contents, special efforts were made to eliminate this error. The values obtained for pigs X and Y are presented in table 4.

TABLE 4. SLAUGHTER TECHNIQUE STUDY LOG OF THE NUMBER OF VARIABLE CELLS PER GRAM DRY STOMACH CONTENTS OF HOGS ANESTHETIZED BEFORE SLAUGHTER

Organisms	Pig X (July 9)	Pig Y (July 26)
Coliforms	6.0	6.9
Enterococci	3.2 (See text)	5.1
Lactobacilli	7.6	7.8
Yeasts	5.4	2.8
Molds	4.3	5.0

Except for the S.F. medium tube loss incurred due to water bath failure, the values for pig X are similar to those obtained by routine methods which did not include the precautions used here. The counts for pig Y were in the same range, and in this case it was felt that all chances of reverse peristalsis or the like had been minimized. Gram stains of the flora in the S.F. medium incubated at 37° C. revealed forms typical of enterococci.

With reference to the apparently inert forms of molds, to which attention was drawn in the data presented in table 3, the only contrary evidence is that of Quinn *et al.* (1953) who found an *Aspergillus flavus* involved in the response of antibiotic-treated pigs, but this may not be pertinent to ordinary diets.

Variation was again generally less than 10^2 except for between year variation in the case of yeasts and molds.

Parakeratosis. Two preliminary studies were also conducted to enumerate the fecal microflora of pigs fed diets high in calcium and containing corn and soybean oil meal, which produced parakeratosis. Bacteriological methods were essentially those described previously, except that coliforms and enterococci were particularly studied since these groups showed considerable change as the treatment period progressed. Zinc therapy was imposed as the lesions developed. In a third trial, high *vs.* low calcium diets were compared for their effects upon the fecal coliform and enterococci counts. In a fourth trial the sole remaining variable, the presence or absence of 5% alfalfa meal, was employed. The reason for this was that the alteration of either the calcium or zinc level of the ration did not restore the flora to a

status equivalent to that of the pigs fed the basal diet of corn, soybean oil meal, and alfalfa meal used in the first experiment.

In the first trial a gradual depression in excess of 10^2 of both coliforms and enterococci developed as the parakeratosis lesions slowly developed in the eight pigs studied. The other organisms studied were not noticeably altered. Fifty p.p.m. zinc, included in the diet as zinc carbonate, was without detectable effect upon the flora. No mediating role can be ascribed to the flora. In a second trial, a similar depression was noted but it was neither as consistent nor as marked as in the first. Variation among individuals exceeded 10^2 and this fact necessitates some qualification of the previous conclusion concerning this range. In a third trial, there were no consistent differences between the flora of the pair of pigs fed a high (about 2%) calcium diet and those fed a low calcium diet; a fourth trial revealed no consistent differences between a pair of pigs fed the diet with 5% ground alfalfa hay and those fed the corn-soybean oil meal basal diet.

Summary

Selective media were employed to enumerate fecal flora of pigs fed four diets. Average bacterial counts obtained were coliform, 10^{10}; enterococci, 10^8; lactobacilli, 10^8; yeasts, 10^4; molds, 10^4; *proteus spp.*, 10^4; clostridia, none. However, clostridia may be present, as indicated by preliminary work by Dr. Helen R. White. The range of variation encountered was consistently less than 10^2, except for yeasts, and this fact was considered to be a reasonable criterion for judging treatment effects.

Selective media were also used to estimate the number of viable organisms in the contents of the intestinal tract of pigs. The site of greatest microbial activity appeared to be the large intestine. The site of least activity appeared to be the stomach but evidence is presented to contradict the traditional concept of sterility or a "germicidal barrier" in the stomach.

Molds were present in the feed and in the contents of the stomach, small intestine, large intestine and feces in nearly the same numbers and are probably there principally as inert transient forms.

Efforts were made to determine the cause of the depression of coliforms and enterococci noted in the fecal flora of pigs fed diets which induce parakeratosis. Zinc, calcium and 5% alfalfa meal were considered as variables but none appeared to be responsible for the floral alteration. In some instances individual variation exceeded the 10^2 limit previously found to describe the "normal" range.

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RATES OF VOLATILE FATTY ACID PRODUCTION IN THE BOVINE RUMEN [1]

W. E. STEWART,[2] DARYL G. STEWART AND L. H. SCHULTZ [3]

Cornell University,[4] Ithaca, New York

IT has been established that the volatile fatty acids which are produced in the digestive tract of the ruminant by microbial fermentation processes represent an important source of energy to the host. The amounts and proportions of the volatile fatty acids (VFA) produced are variable, depending on the nature of the diet, the time after feeding and the age of the animal.

The concentrations of VFA in the reticulo-rumen at various times after feeding are of interest but do not necessarily represent their daily production. Experiments have been conducted on the absorption of VFA from the reticulo-rumen (McAnally and Phillipson, 1943; Barcroft *et al.*, 1944; Danielli, *et al.*, 1945; Gray, 1947, 1948; Masson and Phillipson, 1951; Kiddle *et al.*, 1951; Pfander and Phillipson, 1953; Johnson, 1951; Flatt *et al.*, 1956), but in general the experimental conditions have not simulated normal conditions sufficiently to allow conclusions to be drawn about the quantities of these acids normally produced and absorbed. The *in vitro* method of Carroll and Hungate (1954) and the rumen perfusion method of McCarthy *et al.* (1957a, 1957b) make possible the study of total VFA production under more natural conditions.

This report describes a procedure developed by the authors for estimating the production, absorption and energy contribution of the VFA produced *in vivo* under essentially normal conditions. The method is similar to that of Carroll and Hungate (1954). The method involves removing a sample of rumen ingesta and incubating it anaerobically for a short time. A curve is constructed from the increase in VFA levels in the sample during the incubation period. The slope of the curve at zero time, which is determined mathematically, is assumed to be the rate of production *in vivo* at the time the sample is obtained.

Experimental Procedure

Sampling. To determine rates of VFA production, representative 350 to 400 ml. samples of ingesta were obtained from the reticulo-rumen of fistulated steers at different times after feeding. The samples were taken

[1] These data were taken from material submitted by W. E. Stewart in partial fulfillment of the requirements for the Doctor of Philosophy degree, Cornell University, Ithaca, New York. 1957.

[2] Present address: Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, New York.

[3] Present address: Dairy Husbandry Department, University of Wisconsin, Madison, Wisconsin.

[4] Animal Husbandry Department.

randomly from the reticulo-rumen except that enough fluid was always included to insure good subsampling. The average dry matter content of these samples would have equalled that for the entire rumen. The sample was immediately subsampled with a 10-ml. pipette, having an enlarged tip, and the jar containing the ingesta was placed in the constant temperature incubation apparatus described by Stewart and Schultz (1958). The jars were covered as previously and gassed with carbon dioxide at a slow rate. Another 10 ml. subsample was taken at 30 and 60 minutes after the initial sample was obtained. All samples for VFA analysis were poisoned with a saturated solution of mercuric chloride at the rate of one part of saturated mercuric chloride solution to fifty parts of sample and refrigerated. Two jars of ingesta were obtained from each steer at each time of sampling and duplicate analyses were made.

A sampling period consisted of 24 hours in which two steers were sampled immediately before feeding and watering and at 1 hour, 2 hours and every 2 hours thereafter. A 3-hour sample was included in the first two 24-hour sampling periods, but since the fermentation peak had passed before the 2-hour sample was taken it was discontinued.

Feeding of Animals. The steers were fed at 8 a.m. and 6 p.m. throughout the course of this experiment. The animals were watered twice daily by bucket so that the water intake could be controlled. Water intakes were recorded for 3 days prior to a sampling period. The water intake at each feeding was limited to 70 lb. so that great fluctuations in consumption could not occur. The total daily water consumption was approximately 125 lb.

Two 1500-lb. Holstein steers were individually fed four different rations during the course of the experiment. The steers were not sampled until they had been on the rations at least 3 weeks. The experimental rations are described in table 1. The four rations were hay-only, hay plus urea, hay plus grain, and hay, grain and urea. Since the steers would not consume the urea unless it was mixed with concentrates, 2 pounds of grain had to be included in the hay plus urea ration. However, in the text this ration will be referred to as the hay plus urea ration. The proximate analysis and digestibility data for the second cutting alfalfa hay fed during part of the experiment are also given in table 1. Hay of comparable quality and type was used throughout this experiment. One-half of each ration was fed at each feeding. Blocks of iodized salt were available at all times. In general the urea decreased the palatability of the concentrates so that some of the hay was often eaten first. Two 24-hour sampling periods were completed for each steer while on each ration. The two steers were sampled on the same days during each period.

Volume of Rumen Contents. The steers were maintained on the hay and hay plus grain rations for an extended period so that the changes in the volume of rumen contents could be determined. During this period they were fed and watered in the same way as during the previous sampling

TABLE 1. EXPERIMENTAL RATIONS AND AVERAGE PROXIMATE ANALYSIS AND DIGESTION COEFFICIENTS FOR SECOND CUTTING ALFALFA HAY[a]

Experimental rations

Ration numbers	1	2	3	4
Second cutting alfalfa hay, lb.	28	26	28	26
16% C.P. dairy concentrate, lb.	—	6	2	6
Urea, lb.[b]	—	—	0.4	0.4

Average proximate analysis and digestion coefficients for second cutting alfalfa hay

Item	Proximate analysis (%)	Average digestion coefficients
Dry matter	89.13	
Protein	19.73	75.4±1.3
Ether extract	1.66	26.7±4.7
Crude fiber	28.60	36.4±2.0
NFE	42.95	74.1±1.1
Ash	7.06	
Organic matter	——	61.0±0.3
Energy		
(Heat of combustion)	4.59 KCAL./gm. DM	59.2±0.3
Digestible energy	2.72 KCAL./gm. DM	

[a] Compiled from David Drori, "Studies in Ruminant Digestion", (unpublished Master's Thesis, Cornell University, Ithaca, 1957).

[b] Urea was mixed with the concentrates, Urea was that sold by Polychemicals Dept., E. I. DuPont de Nemours and Co., Wilmington, Delaware and had a guaranteed minimum nitrogen content of 42%.

period. At various times after feeding the total volume was determined by removing the total reticulo-rumen contents via fistula, weighing them and then sampling them for dry matter. About 80% of the ingesta was then returned to the rumen.

The total volume of water in the rumen at various times was plotted graphically so that its volume at intermediate times could be estimated.

VFA Analysis. The VFA were chromatographically determined by the Keeney (1955) procedure with minor modifications. The analysis was made on 2 ml. of the supernatant solution from centrifuged rumen samples. Centrifuging reduced the variations in titration end-points presumably caused by plant pigments eluted from solid material.

Estimation of Rate of VFA Production. The rate of VFA production in the rumen at the time of sampling was determined by calculating the slope of the *in vitro* VFA production curve at time zero, the instant of removal from the rumen. Since the data followed a curvilinear pattern rather than a linear one, the curve during the first hour was assumed to be described by the quadratic equation, $y=A+Bx+Cx^2$, where y was the increase in mg./100 ml. of VFA over the initial level and x was the time in hours after removal of the sample from the rumen.

This equation was fitted to the three experimentally determined points, (x_1, y_1), (x_2, y_2) and (x_3, y_3).

x_1=time of removal from the rumen, assigned a value of zero; y_1= initial VFA level, assigned a value of zero; x_2=one-half hour after x_1; y_2=increase over initial VFA level up to time x_2; x_3=one hour after x_1; y_3=increase over initial VFA level up to time x_3

Since the slope of the curve, the derivative of y with respect to x, equals B where x equals zero, and since y_1 was assigned a value of zero, the following working formula was derived:

$$B=4y_2-y_3.$$

This was the working formula used in calculating the rate of production in the rumen at the various times after feeding. The rate (B) per hour per unit volume was obtained by multiplying the average 30-minute fatty acid increase (y_2) in the artificial rumen by 4 and then subtracting the total increase in one hour (y_3).

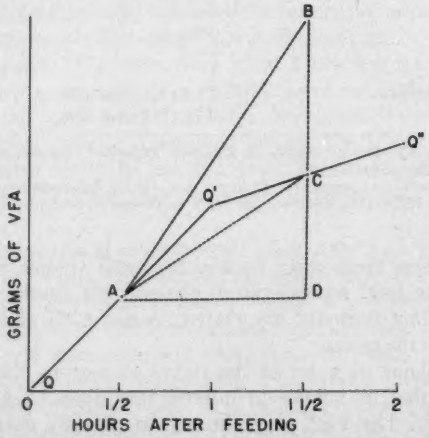

Figure 1. Method of calculating rate of absorption of total VFA from rumen at different times after feeding, using the interval from ½ to 1½ hours after feeding as an example.

Q, Q′, Q″=Total quantity of VFA in the rumen at 0, 1, and 2 hours after feeding, respectively.

A=Total quantity of VFA in the rumen at ½ hour after feeding, determined by averaging Q and Q′.

C=Total quantity of VFA in the rumen at 1½ hours after feeding, determined by averaging Q′ and Q″.

Slope of AB=The rate of total production of VFA in the rumen, which was determined at Q′.

CD=Change in total VFA in the rumen during the hour.

BD=Total production of VFA in the rumen during the hour.

BC=BD−CD, total absorption of VFA from the rumen during the period from ½ to 1½ hours after feeding.

By multiplying a given VFA production rate by the total volume of water in the rumen at that time, the total *in vivo* production rate for that time was obtained. The production rates obtained were assumed to apply to the period from one-half hour before to one-half hour after the time the sample was obtained from the rumen. The total 24-hour production of VFA was obtained by adding the hourly total production rates. An estimate of volatile fatty acid absorption at various times was made, assuming

TABLE 2. OVERALL AVERAGE CONCENTRATIONS AND TOTAL QUANTITIES OF VFA IN THE RUMEN AT DIFFERENT TIMES AFTER FEEDING

	Hours after morning feeding[a]						Hours after evening feeding[a]								
	0	1	2	4	6	8	0	1	2	4	6	8	10	12	Av.	% of Total
Acids[b]	Average concentration for 8 determinations on each of 4 rations (mg./100 ml.)															
V	39	42	50	49	43	40	36	41	51	49	48	44	41	42		
B	126	118	128	137	141	135	129	129	143	154	157	153	139	131		
P	124	131	153	157	154	144	134	151	175	179	181	172	148	138		
A	429	419	471	505	511	495	454	477	537	574	587	570	501	464		
T	718	710	802	848	849	814	753	798	906	956	972	939	829	775		
	Average percent of total acids															
V	5	6	6	6	5	5	5	5	6	5	5	5	5	5	5.2	
B	18	17	16	16	17	16	17	16	16	16	16	16	17	17	16.5	
P	17	18	19	18	18	18	18	19	19	19	19	18	18	18	18.3	
A	60	59	59	60	60	61	60	60	59	60	60	61	60	60	60.0	
	Average total grams of VFA in the rumen[c]															
V	32	41	48	45	37	33	28	45	55	53	49	42	38	36	42	5.2
B	103	117	123	125	122	111	100	141	154	164	161	149	128	114	130	16.4
P	102	129	147	144	134	119	104	164	190	190	186	168	137	120	146	18.4
A	350	413	454	462	444	407	352	520	581	611	602	556	463	404	477	60.0
T	587	700	772	776	737	670	584	870	980	1018	998	915	766	674	795	

[a] Steers were fed at 8 a.m. and 6 p.m.
[b] V=valeric, B=butyric, P=propionic, A=acetic, and T=total.
[c] Total grams obtained by multiplying concentration by volume of rumen contents.

that the total absorption of VFA from the rumen during a given one-hour period equalled the total production of VFA in the rumen during that period, minus the change in total quantity of VFA in the rumen during that period. Figure 1 illustrates this method of calculating total absorption of volatile fatty acids from the rumen for a given hour, in this case, ½–1½ hours after feeding.

pH Determination. During some of the sampling periods a sample of rumen fluid was obtained in a covered beaker at the same time as the samples for incubation were obtained. These determinations were completed within one minute, using a Beckman pH meter.

Results and Discussion

Variations in the Concentrations of VFA in the Rumen. The average concentrations of the various VFA in the rumen at different times after feeding are given in table 2. The VFA concentrations increased after

feeding in all cases. The concentrations reached a higher peak after the evening feeding than after the morning feeding. After the 4- to 6-hour peak the VFA concentrations in the rumen decreased rapidly, as has been reported by many workers. The average concentrations by weight were 5.2% valeric, 16.5% butyric, 18.3% propionic and 60.0% acetic. In general, the relative proportions of the VFA in the rumen stayed remarkably constant.

Rates of VFA Production in the Rumen at Different Times. Table 3

TABLE 3. OVERALL RATES OF VFA PRODUCTION AND TOTAL PRODUCTION OF VFA IN THE RUMEN AT DIFFERENT TIMES AFTER FEEDING

	Hours after morning feeding[a]						Hours after evening feeding[a]						
	0	1	2	4	6	8	0	1	2	4	6	8	10	12
	Average rate of production for 8 determinations of each of 4 rations (mg./100 ml./hr.)													
Acids[b]														
V	2.3	11.5	10.9	0.7	4.8	0.7	5.6	13.8	9.6	5.7	4.2	3.5	3.4	−1.0
B	13.1	31.0	31.3	17.1	16.8	16.8	27.5	31.9	26.6	30.4	26.2	15.7	20.9	23.4
P	13.6	52.6	36.8	27.1	12.0	22.8	23.1	34.6	22.8	33.0	25.3	16.9	24.8	15.2
A	47.1	112.2	117.0	87.0	60.5	52.1	97.0	103.4	78.3	85.2	77.6	51.0	79.8	66.2
T	76.1	207.3	196.0	131.9	94.1	92.0	153.2	183.7	137.3	154.3	133.3	87.1	128.9	103.8
	Average total production per hour (gm.)[c]													
V	2	11	10	1	4	−1	4	15	10	6	4	3	3	−1
B	11	31	30	16	15	14	21	35	29	32	27	15	19	20
P	11	52	35	25	10	19	18	38	25	35	26	16	23	13
A	38	111	113	80	53	43	75	113	85	91	80	50	74	58
T	62	205	188	122	82	75	118	200	149	164	137	84	119	90

[a] Steers were fed at 8 a.m. and 6 p.m.
[b] V=valeric, B=butyric, P=propionic, A=acetic, and T=total.
[c] Average total production obtained by multiplying rate of production by volume of rumen contents.

contains the average data on the calculated rates of VFA production in the rumen at different times after feeding. The maximum rate of VFA production probably occurred within one hour after feeding. A faster rate of production occurred for each acid after the morning feeding, but the production rates also declined faster in the morning. Possibly the lower initial end product concentration contributed to this faster rate of production, since 14 hours had elapsed since the previous feeding.

The proportion of propionic acid in the rumen always increased slightly immediately after feeding, as was first reported by Gray and Pilgrim (1951), but it was not known previously whether this was because of a slower rate of absorption or a greater rate of production. The data show that propionic acid was produced at a faster rate than butyric or acetic acids immediately after feeding. Valeric acid was also produced more rapidly, which probably reflects the rapid rate of protein deamination, shown by Shazly (1952) to occur in the rumen at this time.

At 10 hours after both the morning and evening feedings there was a prominent increase in acetic acid production, with the other acids showing

only slight changes. This increased rate of acetic acid formation subsided by 12 hours after feeding. This phenomenon occurred after each feeding with all rations. A possible explanation is that cellulose digestion apparently is not extensive until 6 to 12 hours after feeding, as shown by Hale *et al.* (1947). However, Belasco (1955) found *in vitro* that the digestion of cellulose yielded more propionic acid and less acetic and butyric acids than digestion of starch.

Volume of Rumen Contents. Eighteen determinations were completed for the hay and hay plus grain rations. Volume of rumen contents increased markedly after the steers were fed and watered, the change being from 180 to 217 and from 171 to 241 lb. of water for the first hour after the morning and evening feedings, respectively. Total volume of water was used in the calculation instead of total contents because the VFA analyses were carried out on the supernatant solution of centrifuged samples.

The percentage of dry matter varied only slightly at various times during the day. The range was from 11.7 to 13.4% in this experiment. The highest percent dry matter, 13.4%, occurred just prior to the evening watering.

Total Quantities of VFA in the Rumen at Various Times After Feeding. By multiplying concentration per unit volume by the volume of water in the rumen at various times during the day, the total amount of VFA in the rumen was calculated. Table 2 contains the overall average data on the total quantities of VFA in the rumen for all four rations. The total quantities of VFA increased abruptly after feeding. However, by eight hours after feeding the total quantities of the VFA were decreasing. Due to the combination of higher concentrations of VFA in the rumen after the evening feeding and greater total volume of rumen contents, the total quantities of VFA in the rumen after the evening feeding were much higher than after the morning feeding.

Total Rumen Production of VFA Per Hour. Table 3 contains the average data on the total production of VFA at various times after feeding for the four rations studied. The peak in total production rate was probably reached within one hour after feeding. Fermentation was still fairly rapid 14 hours after feeding, for the rate of VFA production was still about one-third of that at one hour after feeding. Previously, Hale *et al.* (1947) concluded from rumen digestion coefficients that ruminal digestion was essentially complete by 12 to 14 hours after feeding.

Total Production in Twenty-four Hours. By adding the total amounts of each acid produced each hour for 24-hours the total production of the various VFA in the reticulo-rumen was obtained for each ration. Since samples were obtained only at 2-hour intervals during most of the 24-hour period, average values were calculated for intermediate hours. Table 4 contains these data for each of the four rations studied. Only slight differences occurred in the total VFA production between the four rations. The hay ration yielded 2.82 kg. of VFA, the hay plus urea 2.87 kg., the

TABLE 4. TOTAL VFA PRODUCTION AND TOTAL CALORIES FROM EACH RATION IN 24 HOURS

Ration number		1	2	3	4	1–4
Constituents		Hay	Hay Grain	Hay Urea	Hay Grain Urea	Av.
				Total production per day (gm.)		
Acids						
Valeric		91	158	132	49	108
Butyric		471	523	509	570	518
Propionic		572	590	515	558	559
Acetic		1688	1673	1717	1894	1743
Total		2822	2943	2873	3071	2927
				Per cent of total		
Valeric		3.2	5.4	4.6	1.6	3.7
Butyric		16.7	17.8	17.7	18.6	17.7
Propionic		20.3	20.0	17.9	18.2	19.1
Acetic		59.8	56.8	59.8	61.6	59.5
	Cal./gm.[a]		Total kcal. obtained in 24 hours from VFA			
Valeric[b]	6.68	.61	1.06	.89	.33	.72
Butyric[c]	5.95	2.81	3.11	3.13	3.39	3.08
Propionic	4.96	2.84	2.93	2.55	2.77	2.77
Acetic	3.49	5.89	5.84	5.99	6.61	6.08
Total		12.14	12.93	12.46	13.10	12.66
				Per cent of total calories		
Valeric		5.0	8.2	7.1	2.5	5.7
Butyric		23.1	24.1	24.3	25.9	24.3
Propionic		23.4	22.6	20.5	21.1	21.9
Acetic		48.5	45.1	48.1	50.5	48.1

[a] Based on heats of combustion given by Lange *et al.* (1939).
[b] Calculated as if entire fraction were n-valeric.
[c] Calculated as if entire fraction were n-butyric.

hay plus grain 2.94 kg. and the hay, grain and urea 3.07 kg. This order is the same as that of the gross energy contents of the rations. The average was 2.93 kg. Small differences between rations also occurred in the proportions of the VFA produced.

Total Absorption of VFA. Since the VFA presumably are not metabolized further in the digestive tract but are absorbed, and since the concentrations of the VFA in the abomasum are low (Phillipson and McAnally, 1942; Gray *et al.*, 1954), their total production during a 24-hour period should equal their total absorption. Thus the values given in table 4 for total production were also considered to be the values for total absorption from the forestomach for a 24-hour feeding cycle.

Relative Rates of VFA Absorption In Vivo. Of the total acids which were produced and absorbed, 3.7% was valeric acid, 17.7% was butyric,

19.1% was propionic and 59.5% was acetic acid (table 4). This compares with an average 24-hour total content of 5.2% valeric, 16.4% butyric, 18.4% propionic and 60.0% acetic acid (table 2). Therefore, there appears to be little or no preferential absorption of the various VFA from the normally functioning rumen, absorption being proportional to concentration. This suggests that the variations in the proportions of the VFA

TABLE 5. PERCENTAGE OF THE DIGESTIBLE ENERGY OF THE RATIONS WHICH WAS SUPPLIED BY THE VFA PRODUCED

Ration number	1	2	3	4	1–4
Constituents	Hay	Hay Grain	Hay Urea	Hay Grain Urea	Av.
Gross energy of rations (therms)[a]	51.99	——	——	——	——
Digested calories from each ration (therms)[b]	29.12	36.30	32.21	36.30	33.48
Caloric value of the VFA produced in the reticulo-rumen (therms)	12.14	12.93	12.46	13.10	12.66
Percent digested calories from VFA	41.7	35.6	38.4	36.1	37.9
Percent of gross energy of the ration appearing as VFA	23.3	——	——	——	——
Percent of maintenance requirement furnished by VFA[c]	——	——	——	——	63.0

[a] Gross energy of the hay was 4.09 cal./gm. Gross energy analyses on the grain and urea were not made.

[b] Digestible energy of concentrates based on "Nutrient Requirements of Domestic Animals. Number III. Nutrient Requirements of Dairy Cattle" revised 1956. National Academy of Sciences-National Research Council. Publication 464, pp. 21–22.

[c] Digestible energy requirement for a 1500 lb. cow for maintenance is 20.1 therms. *ibid.*, p. 4.

which occur with time after feeding and with different types of diets are the results of differential rates of production, not absorption.

Caloric Value of the VFA Produced and Absorbed. The caloric values of the VFA produced and absorbed, which are the products of the amounts of the individual acids produced and their heats of combustion, averaged 12.7 therms for the four rations (table 4). Of this, the valeric acid fraction provided 5.7%, calculated as n-valeric; butyric acid, 24.3%, calculated as n-butyric; propionic acid, 21.9% and acetic acid 48.1%.

As the estimated digested calories of the various rations increased from 29.1 to 36.3 therms, the caloric value of the VFA produced increased from 12.1 to 13.1 therms (table 5). However, the increase in VFA production per unit increase in digested calories occurred at a diminishing rate. The

VFA accounted for an average of 38% of the digested calories of the rations. The largest percentage, 42%, was obtained with the hay ration. Twenty-three per cent of the gross energy of the hay was accounted for by the VFA produced in the reticulo-rumen.

If the National Research Council recommendations for dairy cow maintenance are assumed to apply to mature dairy steers as well, the VFA produced in these experiments would account for 63% of the 20.1 therms required for a 1500 lb. steer. McCarthy *et al.* (1957a), using their rumen perfusion technique, estimated that rumen VFA would provide 37 to 46% of the energy requirement of the goat for maintenance.

TABLE 6. RATES OF VFA ABSORPTION AND pH CHANGES WITH TIME

Time after morning feeding[a] (hours)	Total VFA absorption during period (gm.)	pH[b]	Time after evening feeding[a] (hours)	Total VFA absorption during period (gm.)	pH[b]
0.0– 0.5	—28[c]	6.83	0.0– 0.5	—84[c]	6.66
0.5– 1.5	111	6.68	1.5– 2.5	85	6.43
1.5– 2.5	152	6.66	2.5– 3.5	138	——
2.5– 3.5	153	——	3.5– 4.5	159	6.39
3.5– 4.5	130	6.53	4.5– 5.5	160	——
4.5– 5.5	116	——	5.5– 6.5	163	6.35
5.5– 6.5	108	6.53	6.5– 7.5	152	——
6.5– 7.5	115	——	7.5– 8.5	143	6.42
7.5– 8.5	114	6.69	8.5– 9.5	177	——
8.5– 9.5	139	——	9.5–10.5	179	6.59
9.5–10.0	82	6.66	10.5–11.5	150	——
			11.5–12.5	135	6.70
			12.5–13.5	120	——
			13.5–14.0	53	6.83

[a] Animals were fed and watered at 8 a.m. and 6 p.m.
[b] Each value is the average of eight determinations.
[c] This value is erroneous. See text.

Rates of Absorption at Various Times After Feeding. The rates of absorption at various times after feeding are given in table 6. The negative absorption values arose because the pre-feeding rate of VFA production was used as the rate of production during the first half hour after feeding. Actually, it was probably higher, since the peak production rate occurred immediately after feeding. However, no samples were taken until one hour after feeding in these experiments. This error caused the total VFA production data to be in error by the same amount, so the production figures presented earlier should be considered as minimal. Except for the fluctuations immediately after feeding, VFA absorption rates tended to be fairly uniform. A slight tendency for absorption to increase when the concentrations of VFA in the rumen were highest (table 2) and rumen pH's

lowest (table 6) was noticeable. Danielli *et al.* (1944) found that absorption rates increased as the pH decreased from 7.5 to 5.8. Also, the absorption rate was somewhat greater after the evening feeding as compared to the morning feeding.

Rumen pH Changes. Rumen pH (table 6) decreased after each feeding, the decrease being greater after the evening feeding, when the VFA concentrations were higher. The lowest average value, 6.35, was obtained 6 hours after the evening feeding. The highest, 6.83, was obtained just before the morning feeding.

TABLE 7. AVERAGE CONCENTRATIONS OF VFA IN THE RUMEN FOR EACH RATION

Ration number	1	2	3	4	1–4
Constituents	Hay	Hay Grain	Hay Urea	Hay Grain Urea	Av.
Acids	Average hourly concentrations through 24 hr. (mg./100 ml.)[a]				
Valeric	50	49	39	39	44
Butyric	124	143	141	146	138
Propionic	147	150	165	157	155
Acetic	489	483	538	515	506
Total	810	825	883	857	843
	Percent of total from each acid				
Valeric	6.1	5.9	4.4	4.6	5.2
Butyric	15.4	17.4	16.0	17.0	16.5
Propionic	18.1	18.2	18.7	18.3	18.3
Acetic	60.4	58.5	60.9	60.1	60.0

[a] Average of four 24-hour sampling periods on two fistulated steers with two replicate samples obtained at each hour.

Relationship Between Average Concentration of VFA and Total VFA Production. Many workers have been studying the effect of different rations on the concentrations of volatile fatty acids in the rumen, and have measured the concentrations at a given time after feeding. In some of the studies statistically significant VFA concentration differences between rations have been found. However, information on VFA concentrations in the rumen adds little to our knowledge of their value to the nutrition of the ruminant unless there is a correlation between their concentration and total production.

Table 7 contains the data on the average 24-hour concentrations of VFA in the rumen for each of the four rations, which were obtained by averaging all the hourly concentrations of VFA. Only slight differences in the average concentrations occurred, the range in average concentrations being from 810 to 883 mg. total VFA/100 ml. Even though the differences

were small, the hay ration had both the lowest average concentration of total VFA, 810 mg./100 ml., and the lowest quantities of total VFA produced in 24 hours, 2,882 gm. This suggests that valid estimates of VFA production differences between rations can be made on the basis of concentration differences if the time at which the samples are taken relative to feeding is controlled. A more detailed study of this with rations of more divergent caloric content is needed.

Comparison of In Vitro and In Vivo VFA Production Results. Urea significantly stimulated VFA production *in vitro,* as reported by Stewart and Schultz (1958), but urea addition to the hay and grain ration did not markedly increase VFA production *in vivo.* This suggests that the course taken by long term *in vitro* incubations is different from that occurring *in vivo,* where the microorganisms are affected by the continuous absorptive and secretive processes of the host.

Summary

By means of an *in vivo-in vitro* technique, volatile fatty acid production rates for four rations fed twice daily to two 1500 lb. fistulated Holstein steers were determined at various times after feeding. Volume of rumen contents changes were also studied. The following results were obtained:

1. The concentration of VFA in the rumen increased after feeding for from 4 to 6 hours.

2. Rates of VFA production were greatest within the first 2 hours after feeding.

3. The rates of production of propionic and valeric acid were proportionately greater than those of the other fatty acids for the first 2 hours after feeding.

4. Volume of rumen contents increased greatly after the animals were fed and watered, but the percent dry matter of the rumen contents varied less than 2%.

5. Volume of rumen contents and the total quantities of VFA in the rumen were greater after the evening feeding.

6. Of the average 2,927 gm. of VFA produced per steer during a 24-hour period, valeric acid amounted to 108 gm., butyric acid 518 gm., propionic acid 559 gm. and acetic acid 1,743 gm.

7. Of the 12.7 therms supplied by these VFA, 5.7% was from valeric acid, 24.3% from butyric acid, 21.9% from propionic acid and 48.1% from acetic acid.

8. As the digested colories of the various rations increased from 29.1 to 36.3 therms the caloric value of the VFA production increased, but at a diminishing rate. The VFA produced in the reticulo-rumen would account for from 36 to 42% of the estimated digested calories of the four

rations studied. These acids, on the average, accounted for 63% of the energy requirements of the steers for maintenance. Twenty-three per cent of the gross energy of the hay ration appeared as reticulo-rumen VFA.

9. No preferential absorption of the various VFA occurred, absorption being proportional to concentration.

10. Whereas only slight differences in the average concentrations of VFA in the rumen occurred between the four rations; the production differences were proportionately greater.

11. Rumen pH decreased after each feeding.

12. Urea did not markedly stimulate VFA production *in vivo* as it did in the previous *in vitro* experiments.

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IN VITRO VOLATILE FATTY ACID PRODUCTION FROM VARIOUS FEEDS BY BOVINE RUMEN MICROORGANISMS [1]

W. E. STEWART [2] AND L. H. SCHULTZ [3]

Cornell University,[4] Ithaca, New York

PROPIONIC acid has been shown to be glucogenic and to relieve the ketotic condition common in high producing dairy cows (Schultz, 1952; 1953). Butyric acid appears to be ketogenic (Schultz *et al.*, 1949). Since many workers have shown that the concentration of volatile fatty acids (VFA) in the rumen is influenced by the ration fed, an experiment was designed to find a feed or combination of feeds which would favorably influence the total production of VFA, especially the proportion of propionic acid, when these feeds were incubated *in vitro* with bovine rumen microorganisms.

Experimental Procedure

In Vitro Incubations. The incubation method used was of a batch type, in which diluted rumen liquor was the inoculum. A battery of 12 large-mouthed pint jars was used for each replication. The jars were fitted with No. 12 three-hole rubber stoppers. One hole served as a carbon dioxide inlet, the second as a gas outlet, and the third as a port for adding the inoculum. Carbon dioxide, which had been humidified by bubbling through water, was bubbled through each jar independently at the rate of 3 to 6 bubbles per second.

The rubber tubing connection to the jars were provided with screw clamps so that the rate of carbon dioxide flow could be adjusted. Inside the jars, microburette tips were attached to the carbon dioxide intake tubing. This constriction of the opening enabled the operator to obtain a uniform rate of gas flow to all 12 jars. The gas outlets had single-bulb glass condensers, 6 to 8 in. above the water bath. Above the bulbs the glass tubing extended another 4 to 6 inches. The condenser bulb minimized the loss of water vapor. The large central opening for adding the inoculum was fitted with a No. 0 rubber stopper. A large thermostatically controlled water bath maintained the temperature of the incubation jars at 39 to 39.5° C.

Charging the Jars. Prior to obtaining the inoculum from the steer, weighted amounts of the various feeds were added to the jars with 200 ml.

[1] These data were taken from material submitted by W. E. Stewart in partial fulfillment of the requirements for the Doctor of Philosophy degree, Cornell University, Ithaca, New York, 1957.

[2] Present address: Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, New York.

[3] Present address: Dairy Husbandry Department, University of Wisconsin, Madison, Wisconsin.

[4] Animal Husbandry Department.

of distilled water and 50 ml. of nutrient salt solution (Wasserman *et al.*, 1952). Then the jars were placed in the water bath and the carbon dioxide connections and adjustments made.

Inoculum. The inoculum was obtained alternately from one of two 1500-pound Holstein steers which were fed alfalfa mixed hay twice a day. One steer also received 2 lb. of corn meal at each feeding. Fresh water and salt were available at all times. Wood shavings were used for bedding. Two liters of inoculum were obtained before feeding from one of the steers via a rumen fistula. The warm inoculum was strained through four layers of cheesecloth and 100 ml. was added to each jar. At the same time, a measured sample of inoculum was poisoned with a saturated solution of mercuric chloride added at the rate of one part of mercuric chloride to 50 parts of filtrate and refrigerated until the volatile fatty acid determinations were made. During the first 4 hours, the lids of the jars were raised occasionally and the floating substrate stirred down to prevent the liquid from rising up the condenser tube into the bulb. After 12 hours a representative sample was taken from each jar, poisoned and refrigerated.

Volatile Fatty Acid Analysis. The VFA were determined by the Keeney (1955) procedure, with minor modifications.

Results and Discussion

Experiment 1. Experiment 1 was a preliminary trial conducted to test the reproducibility of the *in vitro* incubation method within a replication. The inoculum was obtained from the steer fed hay and corn meal. One control flask, which contained only diluted inoculum, and 11 treated jars, which each had 10 gm. of corn meal as the only substrate, were tested. In this experiment the jars were sampled at 8 and 12 hours.

The concentrations of each of the fatty acids increased in a linear manner in the control jar, but the magnitude of the increase was only about one-fourth that of the corn meal treated jars. In the treated jars the 12-hour concentrations of VFA were only slightly higher than the 8-hour concentrations (table 1), which indicated that something was inhibiting VFA production, for considerable amounts of residual substrate remained. Every corn meal jar had a lower concentration of the valeric acid fraction at 12 hours than at 8 hours, which indicated that this fraction was being utilized by the microorganisms.

The uniformity of the data was encouraging and prompted further use of this *in vitro* procedure with other feeds.

Experiment II. Hay, Concentrates and Urea. Twelve rations were compared in which 10 gm. of second cutting alfalfa hay, which contained 89.13% dry matter, 17.59% protein, 1.48% ether extract, 25.49% crude fiber, 38.28% NFE, and 6.29% ash, was the only roughage and in which corn meal (5 gm.), beet pulp (5 gm.), urea (1 gm.) and molasses (1 gm.),

TABLE 1. AVERAGE VFA LEVELS IN ELEVEN REPLICATE JARS CONTAINING 10 GRAMS OF CORN MEAL AND DILUTED INOCULUM

Acid	Initial level	Time after inoculation			
		8 hours		12 hours	
		(mg./100 ml.)			
Valeric	17.7	29.8[a]	3.2[b]	24.8[a]	2.4[b]
Butyric	35.0	69.0	5.4	72.0	6.9
Propionic	39.8	70.9	4.7	81.0	4.0
Acetic	122.5	166.2	4.8	175.1	4.2
Total	215.0	335.9	14.5	352.9	14.0

[a] Arithmetic mean.
[b] Standard deviation, ±.

used individually and in various combinations were the concentrates (table 2). Each ration was randomly assigned to a jar for each of four replications of this experiment. The hay was ground through the 2-mm. sieve of a Wiley mill.

Experiment III. Hay and Concentrates with Soybean Oil Meal or Urea. Twelve more rations were compared in four replications with either soybean oil meal or urea (table 2).

Experiment IV. Grass, Concentrates and Urea. Forty grams of fresh finely-chopped, legume mixed pasture grass, which averaged 17.60% dry

TABLE 2. INGREDIENTS USED IN THE VARIOUS RATIONS IN EXPERIMENTS II, III, AND IV

Ration[a]	Expt. II	Expt. III	Expt. IV
1	HU	H	G
2	HUM	Hs	GM
3	HUC	HS	GC
4	HUCM	HBS	GCM
5	HUB	HBSM	GB
6	HUBM	HCS	GBM
7	H	HCSM	GU
8	HM	HcbS	GUM
9	HC	HcbSM	GUC
10	HCM	HcbU	GUCM
11	HB	HcbUM	GUB
12	HBM	Hcbsu	GUBM

H=10 gm. alfalfa hay
C= 5 gm. corn meal
c= 2.5 gm. corn meal
M= 1 gm. molasses
B= 5 gm. beet pulp
b= 2.5 gm. beet pulp
S= 2 gm. SOM
s= 1 gm. SOM
U= 1 gm. urea
u= 0.5 gm. urea
G=40 gm. fresh grass

[a] Four replicates were run of each ration in each experiment.

matter, 5.35% protein, 0.84% ether extract, 3.31% crude fiber, 6.59% NFE, and 1.51% ash, replaced the hay. Otherwise the amounts and types of concentrates (table 2) and the general procedures paralleled Experiment II.

Close agreement between the replicates in each of the experiments was evident except with the grass (Experiment IV). A summary of the results is given in table 3. The effect of a given feed ingredient was determined by comparing its VFA production to that of its control, which had equal amounts of all feed ingredients except the one being tested.

Urea caused the greatest increases in VFA production in all three experiments. However, in subsequent experiments *in vivo* (Stewart *et al.*, 1958) urea did not markedly affect VFA production, although it is recognized that proportionally smaller amounts of urea were used *in vivo*.

Molasses consistently depressed acetic acid production, and corn meal significantly increased propionic acid production (Experiments II and IV). Beet pulp greatly increased acetic acid and, to a lesser extent, propionic acid production, but it did not increase the amounts of butyric and valeric acids (Experiments II and IV). Belasco (1955) reported that *in vitro* urea utilization and VFA production were generally higher with starch than with cellulose, yet in our experiments corn meal and urea consistently yielded lower levels of VFA than beet pulp and urea.

Hay did not yield as large quantities of VFA as did grass, even though the grass supplied less total dry matter. Grass caused the production of less propionic acid, which is in agreement with the *in vivo* work of Card and Schultz (1953), in which they compared the rumen VFA concentrations of cows fed hay and those on pasture.

Contrary to findings of Emery *et al.* (1956) inoculum source did not significantly affect individual or total VFA production (table 3). Comparison of the *in vitro* data presented in this paper with the subsequent *in vivo* data (Stewart *et al.*, 1958) in respect to the production of VFA on diets made up of hay and/or grain and/or urea indicates that the proportions of acids produced on the two types of experiments are not comparable. All the *in vitro* experiments gave much higher proportions of propionic acid and lower proportions of acetic acid than were produced *in vivo*. Also, urea did not have as marked an effect *in vivo* as *in vitro*. This is not surprising, considering the differences between the conditions of the two experiments. Differences in buffering systems, removal of end products, rate of addition of substrate to inoculum, suitability of inoculum to substrate, and relative proportions of substrate and inoculum are just a few of the factors which could cause differences in the two types of experiments. One must conclude that this *in vitro* method is not a good technique for estimation of total production or proportions of VFA produced *in vivo*.

TABLE 3. COMPARISONS OF THE 12-HOUR *IN VITRO* VFA PRODUCTION FROM VARIOUS FEEDS IN MG./100 ML.

No. of paired rations[a]	Experiment number	Feeds compared	Acetic	Propionic	Butyric	Valeric	Total VFA
	II & III	10 gm. hay only (average totals)	132	116	31	6	285
6	II	Hay and concentrates vs. same plus 1 gm. urea[b]	+180**	+120**	+43**	+12**	+355**
6	II	Hay and concentrates vs. same plus 1 gm. molasses	−23*	−1	−2	−1	−27
4	II	Hay and concentrates vs. same plus 5 gm. corn meal	+5	+40*	+3	−2	+46
4	II	Hay and concentrates vs. same plus 5 gm. beet pulp	+74**	+30	+1	−3	+102**
2	III	Hay and conc. with 2 gm. SOM vs. hay & conc. with 1 gm. urea	+225**	+168**	+59**	+11**	+462**
3	III	Hay and conc. with 2 gm. SOM vs. same plus 1 gm. molasses	−47**	−62**	−6	+2	−113**
1	II, III, IV	10 gm. hay only vs. 40 gm. grass only	+51*	−23*	+48**	+27*	+103*
6	IV	Grass and concentrates vs. same plus 1 gm. urea	+120**	+55**	+47**	+14**	+236**
6	IV	Grass and concentrates vs. same plus 1 gm. molasses	−13	+12	−10	−5	−16
4	IV	Grass and concentrates vs. same plus 5 gm. corn meal	+1	+38*	+8	−7	+40*
4	IV	Grass and concentrates vs. same plus 5 gm. beet pulp	+127**	+41*	−9	−8	+151**
24[c]	II, III	Inoculum from hay vs. hay plus corn meal fed steer	+7	+3	+4	−1	+13

[a] Each pair of rations had equal amounts of all basic feed ingredients except the feed being compared.
[b] Rations with urea increased acetic acid production 180 mg./100 ml. over that of hay and concentrates, etc.
[c] Twenty-four rations were fermented with inoculum from two sources.
* Significant at the 5% level.
** Significant at the 1% level.

Summary

Several common dairy feeds were compared based on their 12-hour production of VFA *in vitro*. The *in vitro* method used yielded reproducible results between replicates.

Urea consistently increased volatile fatty acid production *in vitro* regardless of the substrates used, although in subsequent *in vivo* experiments it increased VFA production only slightly.

Fresh hand-clipped legume mixed grass caused a greater VFA production than did legume hay. The grass markedly depressed propionic acid formation, however, compared to hay.

Molasses consistently decreased acetic acid production. Beet pulp significantly increased acetic acid production and corn meal increased propionic acid production.

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ESTIMATES OF SOME SOURCES OF VARIATION IN THE BODY WEIGHTS OF CROSSBRED LAMBS AT DIFFERENT AGES

R. B. HARRINGTON,[1] J. V. WHITEMAN AND R. D. MORRISON [2]

Oklahoma Agricultural Experiment Station,[3] Stillwater

THE body weight of a lamb is influenced by some non-genetic sources of variation. Adjustment formulas have been or can be devised to correct a weaning weight to a standard condition for lamb or ewe evaluation. Hazel and Terrill (1945, 1946) reported that from 33 to 49.5% of the variation in the weaning weights of lambs reared under range conditions could be accounted for by differences in sex, age of dam, type of birth and rearing, age at weaning and the percent inbreeding. Blackwell and Henderson (1955) analyzed the weaning weights of lambs reared under farm flock conditions, and found differences in sex, breed, age of dam, type of birth and rearing and the age at weaning to be significant sources of variation. deBaca *et al.* (1956) studied the effects of breed of sire, breed of dam, breed of sire $\times$ breed of dam interaction, birth type, sex, and birth type $\times$ sex interaction on the 120-day weights of crossbred lambs. From 45 to 70% of the variation was associated with these variables and increased to 68 to 78% when the effect of birth weight was included.

This paper reports the results of a study designed to determine if some of these variables contribute to variation in 120-day lamb weights under fall lambing conditions to an extent comparable to those reported under other conditions. The study also included estimates of the magnitude of these variables at 15-day age intervals between 45 and 135 days to determine their relative importance as the lamb grows.

Methods and Materials

The lamb weights used in this study were obtained from an experimental sheep flock at the Ft. Reno Experiment Station. The lambs were born in the late falls of 1955 and 1956 and were unselected. The dams of these lambs were grade Rambouillet and grade Rambouillet X Panama-Rambouillet ewes which were purchased as yearlings during April and May 1955 in the Del Rio, Texas, area. All of the lambs were sired by purebred Dorset rams, which were purchased from private breeders in Oklahoma. The ewes were first bred as yearlings during late May, June

[1] Present address: School of Wool Technology, The New South Wales University of Technology, Darlinghurst, New South Wales, Australia.
[2] Department of Mathematics, Oklahoma State University.
[3] In cooperation with Sheep, Goat and Fiber Research Section, Animal and Poultry Branch, A.R.S., U.S.D.A.

and early July 1955 and bred again during late May, June and early July 1956 as two-year-olds.

The flock was managed according to the usual practice of commercial breeders in Oklahoma. During the winter months the ewes were grazed on wheat pasture and received supplemental alfalfa hay during inclement weather. After lambing, the flock was divided into bands, one band made up of ewes rearing lambs and one band of ewes not rearing lambs. The lactating ewes received one-half to one pound of grain daily during the first two to three months of lactation. During the 1956–57 season the ewes rearing twins were separated from those rearing singles and received

TABLE 1. BREEDING, SEX, TYPE OF BIRTH AND REARING AND YEAR OF BIRTH

Breeding [a]	Sex	Type of: Birth	Rearing	1955–56	1956–57
D X RPR	Male	Single	Single	15	21
		Twin	Single	4	3
		Twin	Twin	7	12
	Female	Single	Single	11	23
		Twin	Single	4	2
		Twin	Twin	3	15
		Single	Twin	0	1
D X R	Male	Single	Single	26	26
		Twin	Single	4	1
		Twin	Twin	6	27
	Female	Single	Single	33	27
		Twin	Single	0	0
		Twin	Twin	6	23
Total				119	181

[a] D×RPR=Dorset×(Rambouillet×Panama-Rambouillet).
D×R=Dorset×Rambouillet.

extra grain but were otherwise treated similarly. All the lambs had free access to creep feed consisting of two parts cracked sorghum (kafir) grain and one part chopped alfalfa hay (good quality). The lambs were separated from their dams only during the time of weighing.

The birth weight of the lambs was recorded to the nearest 0.5 lb. in 1955 and to the nearest 0.1 lb. in 1956. The lambs were weighed at approximately two-week intervals commencing when the older lambs in the flock were about 40 days old and continuing until the lambs reached a market weight of approximately 90 lb. Each lamb was identified by a metal ear tag and a number painted on his back. All lambs were docked

during the first week after birth. The ram lambs were castrated between 1 and 4 weeks of age.

The distribution of these lambs according to breeding, sex, type of birth and rearing and year of birth is presented in table 1. The weights of the lambs were adjusted to the following constant ages: 45, 60, 75, 90, 105, 120 and 135 days. The study was not continued beyond 135 days of age since some of the lambs had already reached market weight and were sold. The method used to adjust the weights to a constant age was the linear interpolation method described by Taylor and Hazel (1955).

The data were analyzed on a within year basis. The least squares method of obtaining constants was used to contend with multiple classification and unequal subclass numbers as outlined by Anderson and Bancroft (1952). Each observation of an age adjusted weight was assumed to be the sum of the influences or effects of the other variables as follows:

$$Y_{ijkm} = M + B_i + T_j + S_k + R_m + WX + e_{ijkm}$$

where

Y_{ijkm} = the age adjusted weight of the lamb.

M = a constant for all lambs; the mean of all ewe lambs born and reared as twins with RPR breed of dam. Other effects (B, T, S and R) were estimated as deviations from this class.

B_i = a constant for the i^{th} breed of dam.

T_j = a constant for the j^{th} birth type (single or twin).

S_k = a constant for the k^{th} sex (male or female).

R_m = a constant for the m^{th} type of rearing (single or twin).

W = a constant for the effect of the lamb's birth weight X, a covariable.

e_{ijkm} = error or failure of the above constants to estimate the age adjusted weight of the lamb.

The parameters in the model above were estimated for each of the different ages within each year. Since the normal equations obtained by least squares procedure were not independent, the effect of Rambouillet X Panama-Rambouillet breed of dam, the twin birth type, the female sex, and the twin rearing type were set equal to zero, thus making it possible to find the constants B, T, S, and R as estimates of differences.

Results and Discussion

Rather apparent differences were noted in the rate of gain of the lambs reared in the two different seasons. During the 1955–56 season, 30–40% of the lambs became stiff for differing lengths of time varying from 2 days to 4 weeks. The stiffness occurred when the lambs were between 60 and 120 days of age. Thus, when the mean lamb growth curve was plotted (figure 1), it took on a non-linear appearance. The varying intensity and duration of the stiffness made it impossible to correct for this effect. During the 1956–57 season the growth curve was essentially linear from birth to 120 days. On an age for age basis, the lambs reared during the 1956–57 season were comparatively heavier than those reared during the 1955–56 season. It is impossible to determine how much of the difference in the

weight of the lambs between seasons is due to the age of dam, the stiffness of the lambs and the effect of year because these effects were confounded. Bonsma (1939), Hazel and Terrill (1945, 1946), Sidwell and Grandstaff (1949) and Blackwell and Henderson (1955) have reported that lambs reared by three-year-old ewes are heavier than lambs reared by two-year-old ewes.

Hammond (1932), Sidwell and Grandstaff (1949) and Blackwell and Henderson (1955) have reported that the year in which the lambs are reared is an important source of variation in their body weights. Bonsma

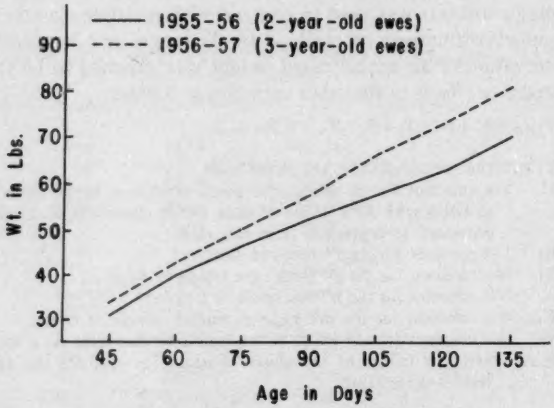

Figure 1. Mean Lamb Growth Curves.

(1939) and deBaca *et al.* (1956) have reported that lamb growth is essentially linear from birth to approximately 135 days of age. This evidence in addition to the fact that lamb growth during the 1956–57 season was essentially linear would lead to the conclusion that the fluctuations in the rate of gain during the 1955–56 season were largely due to the stiffness that affected some of the lambs.

Simple correlation coefficients were calculated between the weights (age corrected only) at different ages and are presented in table 2. The correlation coefficients between weights at adjacent constant ages ranged from 0.91 to 0.98 in 1955–56 and from 0.97 to 0.99 in 1956–57. Correlations between adjacent constant ages would be expected to be high since the same unadjusted weight may have been used to calculate adjusted weights at two different ages and all correlations are part-whole correlations which are automatically correlated to a varying extent. The failure of these correlation coefficients to be unity indicates the combined extent that weighing errors, variation in fill and non-linear growth affected the weights used in the analysis. Correlations between weights at distant

ages may be of some value in a selection program. Correlation coefficients between birth weight and subsequent weights declined from 0.58 to 0.48 in 1955–56 and from 0.66 to 0.57 in the 1956–57 season. The correspondingly higher correlations noted during the 1956–57 season indicates a better linear relationship between age and weight during that growing season.

Estimated Parameters. A particular constant B, T, S, or R can be interpreted as the additive effect on body weight due to the corresponding

TABLE 2. SIMPLE CORRELATION COEFFICIENTS BETWEEN WEIGHTS AT DIFFERENT AGES

Weight at:	45 days	60 days	75 days	90 days	105 days	120 days	135 days
				1955–56 Season (119 lambs)			
Birth	.58	.54	.53	.50	.52	.48	.49
45 days		.96	.90	.81	.75	.69	.69
60 days			.93	.85	.78	.71	.72
75 days				.91	.82	.75	.74
105 days					.91	.84	.81
120 days						.94	.91
135 days							.98
				1956–57 Season (181 lambs)			
Birth	.66	.62	.60	.59	.59	.57	.58
45 days		.98	.96	.94	.92	.90	.90
60 days			.98	.96	.94	.93	.91
75 days				.97	.96	.95	.93
105 days					.98	.97	.96
120 days						.98	.98
135 days							.99

variable. The coefficient, W, of birth weights, X, can be interpreted as the linear rate of change in body weight for each unit change in birth weight. It should be kept in mind when considering the estimates of the effects of breed of dam, birth type, sex, type of rearing and birth weight on the lamb weights at different ages that there is a high correlation between the weights at different ages. This is due to the fact that the same lambs were used within each season to estimate the parameters for the sources of variation studied. These estimates and their standard errors are presented in table 3.

The effect of breed of dam on the lamb weight at different ages was estimated as the difference between the Rambouillet and the Rambouillet X Panama-Rambouillet ewes. During the 1955–56 season, the Rambouillet ewes reared heavier lambs whereas in the 1956–57 season, the lambs reared by the Rambouillet X Panama-Rambouillet ewes were heavier. In the latter season the differences were far from significant.

The effect of birth type was estimated as the difference between the lambs born as singles and the lambs born as twins. This difference is actually the difference between single lambs reared as singles and twin lambs reared as singles. During the initial ages of the 1955–56 season the difference was in favor of the single birth type. At 90 days of age the twin birth type surpassed the single birth type and was 3.0 lb. heavier

TABLE 3. THE ESTIMATED CONSTANTS (DIFFERENCES) AND THEIR STANDARD ERRORS FOR SOURCES OF VARIATION IN THE WEIGHTS OF LAMBS DURING 1955–56 AND 1956–57

Season	Age in days	Mean [a]	S.E. [c]	Breed of dam [b]	S.E.	Birth type (Single minus twin)	S.E.	Sex (Male minus female)	S.E.	Type of rearing (Single minus twin)	S.E.	Birth weight (Partial reg. coef.)	S.E.
1955–56	45	13.9	1.48	1.2	0.75	1.4	1.23	1.4	0.72*	5.7	1.38**	1.5	0.20**
	60	20.6	1.90	1.6	0.97	1.5	1.58	1.7	0.92	6.6	1.77**	1.6	0.26**
	75	25.2	2.27	2.6	1.16*	0.7	1.89	2.1	1.10	7.5	2.12**	1.9	0.31**
	90	30.3	2.78	3.7	1.42*	−1.0	2.31	1.8	1.35	7.8	2.59**	2.1	0.38**
	105	34.0	3.00	2.6	1.53	−2.4	2.49	2.3	1.45	8.3	2.80**	2.4	0.41**
	120	42.6	3.25	1.5	1.66	−2.8	2.70	2.9	1.58	6.7	3.03*	2.4	0.45**
	135	49.0	3.45	1.3	1.76	−3.0	2.87	3.6	1.67*	6.7	3.22*	2.6	0.47**
1956–57	45	15.5	1.43	−0.1	0.66	2.9	1.75	1.9	0.66**	2.6	1.72	2.0	0.21**
	60	22.6	1.78	−0.1	0.82	2.4	2.18	2.5	0.82**	3.8	2.14	2.1	0.26**
	75	29.2	1.99	−0.1	0.91	3.2	2.43	3.3	0.92**	3.8	2.39	2.2	0.29**
	90	35.8	2.21	−0.0	1.02	2.8	2.70	3.5	1.02**	4.3	2.65	2.3	0.32**
	105	42.6	2.43	−0.1	1.11	3.1	2.97	4.4	1.12**	2.6	2.92	2.6	0.36**
	120	49.2	2.67	−0.3	1.22	1.3	3.26	4.4	1.23**	4.4	3.20	2.8	0.39**
	135	54.7	2.76	−0.2	1.27	0.9	3.37	5.1	1.27**	4.6	3.31	3.0	0.41**

[a] Estimated weights of a ewe lamb from Rambouillet × Panama-Rambouillet ewe, born and reared as a twin and with an average birth weight for lambs of the same classification.
[b] Rambouillet minus Rambouillet × Panama-Rambouillet.
[c] Standard errors.
* $P<0.05$.
** $P<0.01$.

at 135 days. The 1956–57 data indicated a difference in favor of the single birth type which fluctuated from approximately 2.4 to 3.2 lb. until the lambs were about 105 days old then dropped sharply. The standard errors of the differences in birth type were comparably larger than those calculated for the differences between breed of dam and sex. Hazel and Terrill (1945, 1946) reported that single lambs were from 2.5 to 5.1 lb. heavier at weaning than twins reared as singles.

The effect of sex was estimated as the difference between males and females. During 1955–56 the males were significantly ($P<0.05$) heavier than the females at 45 and 135 days. There was a general trend towards an increased difference in favor of the male lambs as weight and age increased. Although the differences were greater at subsequent ages than at 45 days, these differences were not significant. The difference between males and females was highly significant ($P<0.01$) at all ages during the 1956–57 season. These differences increased steadily from

1.9 lb. at 45 days to 5.1 lb. at 135 days. The difference in the 1956–57 data at 120 days was in close agreement with the difference of 4.3 lb. in favor of the males at weaning age reported by Blackwell and Henderson (1955). The estimates reported by Hazel and Terrill (1945, 1946) were considerably larger, ranging from 8 to 10 lb. in favor of the males at 120 days under range conditions, however, their estimates were not corrected for birth weight and their males were ram lambs.

During the 1955–56 season the type of rearing was a very important source of variation in the lambs' weights. The lambs reared as singles were significantly heavier than the lambs reared as twins at all ages. Type of rearing was less important during the 1956–57 season. It is felt that this difference between seasons is real even though it is not significant. In the latter season the ewes rearing twins were given additional grain and all ewes were one year older.

TABLE 4. COEFFICIENTS OF DETERMINATION (R^2) OF WEIGHTS AT DIFFERENT AGES WITH BREEDING OF DAM, BIRTH TYPE, SEX, TYPE OF REARING AND BIRTH WEIGHT

Season	Age (days) 45	60	75	90	105	120	135
1955–56	.58**	.52**	.48**	.38**	.37**	.29**	.30**
1956–57	.59**	.53**	.52**	.49**	.46**	.43**	.44**

** $P<0.01$.

Difference in birth weight was the largest single source of variation of any of the factors measured. To estimate the birth weight effect, the lamb's birth weight was used as a covariable. The regression of subsequent weights on birth weight was highly significant ($P<0.01$) at all ages during both seasons. The regression coefficients ranged from 1.5 to 2.6 and from 2.0 to 3.0 in 1955–56 and 1956–57, respectively. In the 1955–56 season the differences in birth weight accounted for 23 to 34% of the variation (r^2) in the age adjusted weights. In 1956–57 differences in birth weight accounted for 33 to 44% of the variation in the lamb weights at different ages. Since birth weight is correlated with the sex and birth type, part of this variation is also associated with these effects. The regression coefficients of subsequent weights on birth weight were about equal to the estimates reported by deBaca *et al.* (1956).

Coefficients of Determination. Coefficients of determination (R^2) of weights at different ages with breeding of dam, birth type, sex, type of rearing and birth weight were found to decrease steadily as the lambs increased in weight (table 4). The coefficients (beyond 60 days of age) obtained during the 1955–56 season were somewhat less than those calculated for the corresponding ages during the 1956–57 season. The stiffness that

effected some of the lambs the first year undoubtedly accounts for some of this difference. These coefficients of determination ranged from 0.58 to 0.29 in 1955–56 and from 0.59 to 0.43 in 1956–57. The coefficients calculated by deBaca *et al.* (1956) were considerably larger but more sources of variation were taken into consideration in their study.

Application of Results. A large portion of the phenotypic expression of a lamb's body weight is due to environmental factors. Consequently, estimates of these environmental factors will enable a breeder to make adjustments for them and improve the accuracy of selection. Under systems of management where the lambs are raised for a fat lamb market, it is necessary to make the selection of replacements before the lambs go to market. The results of this study indicate that the magnitude of some of the environmental effects studied change as the lambs grow older and heavier. Therefore, if a breeder used the same correction factors for lambs differing in age, error could be introduced. For example, using the 1956–57 data, the difference between males and females increased steadily from 1.9 lb. at 45 days to 5.1 lb. at 135 days.

The effects of type of birth and rearing become relatively less important as a lamb grows older. When selecting lambs for replacements, the best results would probably be obtained with selection at older ages when the maternal influence of the dam is less important if the gaining ability of the lamb is the principal criterion of selection. The lamb's weight at a later age may be a better indication of its own ability to grow. Estimates of the heritability of body weight at different ages are needed to answer this question.

Selection of lambs within a flock in only one of the uses of these correction factors. Some recent work by Barnicoat *et al.* (1956) indicates that the milk production of the ewe can be estimated fairly accurately on the basis of the amount of body weight gain of her offspring between birth and six weeks of age. To use this method it is necessary to make adjustments between twin and single lambs. Such corrections would increase the repeatability of milk production as measured.

If more than one ram is used in the flock, it is possible to compare the rams on the basis of their progeny. Since the rams will not sire the same number of males and females or twins and singles, it would be helpful to make adjustments for these differences.

Summary

The weights of 300 Dorset X Rambouillet or Dorset X Rambouillet-Panama-Rambouillet crossbred lambs reared in the winters of 1955–56 and 1956–57 were adjusted to constant ages of 45, 60, 75, 90, 105, 120 and 135 days. The effects of breed of dam, birth type, sex, type of rearing and birth weight on the variation in body weight at different ages were

estimated by least squares analysis. The data were analyzed on a within-year basis because the same ewes were bred each season and the effects of age of dam and year could not be separated.

The results indicate that there was little difference in the rate of gain of the lambs due to differences in the breed of their dams. Differences in lamb weights due to birth type and type of rearing became relatively less important as the lambs grew older. Type of rearing differences were less important among lambs during 1956–57. Male lambs were heavier than female lambs at all ages during both seasons and maintained the same relative importance. Birth weight was the most important source of variation in the lamb weights at different ages. Regression of subsequent weights on birth weight increased steadily from 1.5 lb. at 45 days to 2.6 lb. at 135 days during the 1955–56 season. The regression of corresponding weights on birth weight in 1956–57 increased from 2.0 to 3.0 lb. at 45 and 135 days, respectively. Birth weight accounted for 34 to 44% of the variation at 45 days and from 23 to 33% at 135 days. Where comparable, the differences due to the various sources of variation were found to be in general agreement with those previously reported by other workers.

Coefficients of determination (R^2) indicated that from 29 to 59% of the variation in the lamb weights at different ages could be accounted for by the factors studied. These coefficients were highly significant ($P<0.01$) at all ages during both seasons. The variation accounted for by these variables became relatively less important as the lambs grew older. This was probably due to the diminishing influence of type of birth and rearing.

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REPRODUCTIVE PERFORMANCE OF SWINE FED CHLORTETRACYCLINE OVER SEVERAL GENERATIONS

R. F. SEWELL AND J. L. CARMON [1]

University of Georgia,[2] Athens

EXTENSIVE research has amply demonstrated beneficial effects of feeding antibiotics to growing swine. Available information on the effect of feeding antibiotics to sows during gestation-lactation on their subsequent reproductive performance is limited.

Carpenter (1950) fed sows an APF supplement which supplied approximately 9.5 mg. chlortetracycline per lb. of feed for 49 to 93 days prior to farrowing and during lactation. Litter size or size and livability of the newly born pigs was not affected. Pigs from the sows receiving the antibiotic were creep-fed a ration containing chlortetracycline during the lactation period resulting in heavier weaning weights than the controls. Carpenter (1951) concluded that the increased weaning weight was the result of the pigs consuming feed containing an antibiotic. Carpenter and Larson (1953) fed chlortetracycline at a level of 20 mg. per lb. of feed to gilts from weaning to 200 lb. and reduced the dosage level to 10 mg. per lb. of feed through two subsequent gestation-lactation periods with no apparent beneficial or detrimental effect on reproductive performance. Wallace (1955) fed an antibiotic to sows through five reproductive cycles and concluded that there was no clear evidence of either detrimental or beneficial effects over this long-time feeding period.

DePape *et al.* (1953) concluded that the addition of an antibiotic to sow rations fed prior to breeding and throughout the gestation-lactation period resulted in no significant improvement in number of pigs farrowed, number farrowed alive, average birth weights, or number of pigs weaned.

Davey and Stevenson (1953) fed various levels of chlortetracycline to two generations of sows and reported little variation with respect to appearance of estrus, conception rate and number of litters farrowed among sows on the various treatments. No significant difference was found in litter size or birth weights of the pigs farrowed; although significantly greater weaning weights were obtained with the pigs from sows fed the antibiotic.

To investigate further the effects of antibiotic feeding on the reproductive performance of sows, the experiment reported here was initiated in the fall of 1953 and continued through the spring of 1957.

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Experimental

Eighteen sows and six gilts of purebred Duroc breeding were divided initially into two comparable groups on the basis of age and ancestry. These groups were allotted at random to treatment. Further to reduce genetic variation between the two groups for the course of the experiment, the first two groups of replacement gilts were selected in littermate pairs from sows in each of the two treatment groups and the pair was divided so that one littermate was assigned at random to each of the respective treatment groups. Selections were made at weaning time so that gilts assigned to the group receiving the antibiotic were grown out on rations containing the antibiotic whereas those gilts being added to the sow group not receiving the antibiotic were grown out on rations not containing an antibiotic. After the first two groups of replacement gilts were added, all subsequent gilt replacements were selected from within treatment groups. The same sire was mated to the sows in each of the two groups during a breeding period. Five different sires were used in the course of the experiment.

All replacement gilts for the group of sows receiving the antibiotic were fed the antibiotic throughout the life-cycle. Gilts in the control group were fed similar rations without an antibiotic throughout the life-cycle with the exception of the pig starter which was creep-fed during the nursing period. The same starter ration, which contained the antibiotic, was fed to the pigs of both sow groups during the nursing period.

Sows and litters in both groups were maintained under comparable management procedures at all times. Pasture feeding was used during the breeding and gestation periods. Efforts were made to provide green forage for grazing at all times. The sows were farrowed in a central farrowing barn equipped with farrowing stalls and heat lamps. Sows and pigs were kept in the barn for a 21-day period at the end of which they were returned to pasture. All male pigs were castrated at 21 days of age. Litters were weaned at 50 to 60 days of age and adjusted to 56-day equivalent weights (Whatley and Quaife, 1937).

The basal ration used in feeding the sows was composed as follows: Ground oats, 30.0%; ground yellow corn, 46.9%; soybean oil meal, 15.0%; cottonseed meal, 6.0%; deflourinated phosphate, 1.0%; limestone, 0.5%; salt, 0.5%; and vitamin supplements which contributed the following per lb. of feed: 2 mg. riboflavin, 4 mg. pantothenic acid, 9 mg. niacin, 10 mg. choline chloride, 60 mcg. folic acid, 10 mcg. vitamin B_{12} and 100 I.U. of vitamin D_2. Chlortetracycline was the antibiotic employed and was fed at a level of 5 mg. per lb. of feed to the sows receiving this supplement. During periods of spares forage on the pasture areas due to drought or freezing, dehydrated alfalfa meal was incorporated into the mixture at a level of 5%. The oats in the basal ration was reduced to 10% and the corn increased correspondingly during the lactation period.

Pigs in both groups were allowed free access to creeps containing the same fortified starter ration beginning at two weeks of age until they were weaned. This starter contained 20 mg. of chlortetracycline per lb.

Criteria used in evaluating the results were number of pigs farrowed (alive and dead), birth weights, 21-day weights, 56-day weights and number weaned.

All data were subjected to analysis of variance in evaluating the results obtained.

TABLE 1. COMBINED SUMMARY OF DATA FOR ALL LITTERS

Items	No antibiotic	Antibiotic
Sows bred	64	72
Litters farrowed	56	65
Av. no. pigs farrowed (dead and alive)	9.32	9.26
Av. no. pigs farrowed alive	8.00	8.63
Av. no. pigs farrowed dead	1.32	0.63
Pigs farrowed dead, %	14.18	6.50
Av. birth weight, lb.	2.80	2.76
Av. 21-day weight, lb.	10.55	10.74
Av. no. pigs weaned per litter	5.77	6.97
Av. weaning weight,[a] lb.	35.44	34.74
Av. litter weight,[a] lb.	204.44	242.09

[a] Adjusted to 56-day equivalent weights.

Results and Discussion

A combined summary of the data from litters of all three generations is presented in table 1. Summaries of the data for each generation are presented in table 2.

Statistical analysis of the data revealed no significant difference in total litter size between the sows of the treated and non-treated groups in any generation or in the combined analysis of all generations. The average difference between the two treatments was 0.06 ± 0.62 pigs in favor of the untreated sows.

In the analysis of the combined data a significant increase in the percentage of live pigs farrowed by sows fed the antibiotic was observed (7.6 ± 3.0) as compared to sows not receiving the antibiotic. The average number of live pigs farrowed per litter was 8.00 and 8.63 for the untreated and treated groups, respectively. There was no significant treatment-by-generation interaction. The largest difference between the two treatments occurred in the first generation (11.38%) and the smallest difference in the second generation (4.49%). The difference in the third generation was 7.96%.

Significant treatment differences were also found to exist in the percentage of pigs weaned. Sows fed the antibiotic weaned a significantly higher percentage (12.1 ± 2.8) of the pigs farrowed alive than did the

TABLE 2. SUMMARY OF DATA FOR 1ST 2ND AND 3RD GENERATION LITTERS

Items	No antibiotic	Antibiotic
First generation		
No. litters farrowed	30	29
Av. no. pigs farrowed (dead and alive)	10.10	10.27
Av. no. pigs farrowed alive	8.40	9.30
Av. no. pigs farrowed dead	1.70	0.97
Pigs farrowed dead, %	16.94	9.36
Av. birth weight, lb.	2.82	2.84
Av. 21-day weight, lb.	11.12	10.90
Av. no. pigs weaned per litter	5.43	7.07
Av. weaning weight, lb.[a]	35.20	33.11
Av. litter weight, lb.[a]	191.14	234.09
Second generation		
No. litters farrowed	14	25
Av. no. pigs farrowed (dead and alive)	9.41	8.44
Av. no. pigs farrowed alive	8.71	8.12
Av. no. pigs farrowed dead	0.70	0.32
Pigs farrowed dead, %	10.29	3.79
Av. birth weight, lb.	2.70	2.79
Av. 21-day weight, lb.	9.90	10.91
Av. no. pigs weaned per litter	6.86	6.76
Av. weaning weight, lb.[a]	35.0	36.66
Av. litter weight, lb.[a]	240.10	247.82
Third generation		
No. litters farrowed	12	11
Av. no. pigs farrowed (dead and alive)	7.06	8.17
Av. no. pigs farrowed alive	6.33	7.90
Av. no. pigs farrowed dead	0.73	0.27
Pigs farrowed dead, %	10.59	3.33
Av. birth weight, lb.	3.14	2.47
Av. 21-day weight, lb.	10.15	9.92
Av. no. pigs weaned per litter	5.25	7.18
Av. weaning weight, lb.[a]	36.64	34.84
Av. litter weight, lb.[a]	192.36	250.15

[a] Adjusted to 56-day equivalent weights.

untreated sows. As with the other variates, there was no significant treatment-by-generation interaction with regard to the percentage of pigs weaned. The order of treatment differences followed the same pattern as before. The differences were 15.08, 7.19 and 13.52% for the first, second and third generations, respectively.

No significant treatment effect was found for birth weights, 21-day weights, or weaning weights. The average litter weights for the sows receiving the antibiotic are somewhat larger than litter weights of the untreated group. This difference is largely due to the greater percentage of pigs weaned in the group of sows fed the antibiotic.

It may be observed that the average number of pigs farrowed per litter decreased in both groups with each succeeding generation. Most of the litters represented in the first generation were from sows which had farrowed previous litters. Fewer sow litters are represented in the second generation data with a greater percentage being obtained from first-litter gilts, whereas all of the litters represented in the third generation are from first-litter gilts. Since sow litters are generally larger than gilt litters, this decrease in litter size as presented in the data would be expected.

While the over-all reproductive performance of the sows employed in this experiment was not particularly outstanding, the data are comparable with those of previous studies of this type (Carpenter and Larson, 1953; DePape *et al.*, 1953). Several cases of postpartum metritis were encountered in each of the treated and untreated groups of sows during the course of the experiment. Treatment of the condition when it occurred was left to the discretion of the attending veterinarian. Ration treatment apparently had no effect on the occurrence of the disease since the incidence was approximately the same in both the treated and untreated groups.

U.S.D.A. (1953) reported increased survival among pigs farrowed by sows which had been fed chlortetracycline during gestation-lactation. The improvement in survival appeared to be due to a reduced incidence of stillbirths and first-week mortalities. In the study reported here, there was a decrease in the number of pigs farrowed dead from sows fed chlortetracycline as compared to the sows in the control group. This decrease in stillborn pigs resulted in a greater survival rate. In addition, there was also an increase in the percentage of pigs farrowed alive that were weaned from the group of sows fed the antibiotic.

The mode of action of the antibiotic in decreasing the percentage of stillbirths and increasing percentage of pigs weaned remains obscure. Data presented by Carpenter and Larson (1953) suggest that chlortetracycline is not transferred through the placenta to the embryonic circulation. Consequently, it appears unlikely that the beneficial effects of antibiotic feeding observed in this study would be due to any direct influence of the antibiotic on the embryonic development, particularly at the relatively low level fed. Although the basal ration employed was formulated to be nutritionally adequate with regard to known requirements for reproduction in sows, the possibility of borderline deficiencies can not be completely ruled out. Assuming a marginal nutritional status, the antibiotic may have exerted its influence through some "sparing" action. A more plausible explanation would appear to be the possibility of the antibiotic exerting some control over disease level.

There was no observed difference in the appearance of estrus, conception rate or general breeding efficiency between the two groups of sows. Breeding efficiency in both groups was considered to be satisfactory.

Summary

Feeding the antibiotic chlortetracycline to sows continuously throughout the gestation-lactation cycle for three generations resulted in an increase in the percentage of live pigs born and an increase in the percentage weaned of the live pigs farrowed. No difference was found in total litter size, birth weights, 21-day weights or weaning weights.

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COMPARATIVE VALUE FOR SHEEP OF SOME PROTEIN SUPPLEMENTS FED AT THREE PROTEIN LEVELS

WALTER R. WOODS, WILLIS D. GALLUP AND ALLEN D. TILLMAN

Oklahoma Agricultural Experiment Station,[1] *Stillwater*

THE results of growth, digestion and nitrogen balance trials reported in a previous paper (Woods *et al.*, 1957) indicated that cottonseed meal was inferior to sesame and soybean oil meals as a protein supplement for lambs. The meals were fed in rations containing 9% protein, and the ratio of gain in weight to protein consumed, protein efficiency ratio, was used as one means of evaluation. The relative merits and limitations of this method of evaluation, particularly as applied to supplements fed at a single level of protein intake, have been discussed by Mitchell (1924, 1944) and by Allison (1955). To obtain more detailed information regarding the utilization of nitrogen from these three oil meals, further digestion and nitrogen balance trials were conducted with the meals fed at three levels of intake represented by rations containing 4, 6 and 8% protein.

Experimental

The soybean oil meal and the cottonseed meal used in these comparisons were obtained locally, the former being a solvent processed product and the latter a hydraulic press product. The sesame meal was obtained from the California Milling Corporation, Los Angeles. The chemical composition of the meals is shown in table 1.

The meals were incorporated in semi-purified rations in which they supplied from 90 to 95% of the total nitrogen. The constant part of each ration, in gm., was composed of purified wood cellulose (BW—20 Solka-Floc), 180; cottonseed hulls, 60; minerals (Thomas *et al.*, 1951), 30; vitamin A and D supplement, 1; and d-alpha-tocopherol acetate, 0.01. The cottonseed hulls improved palatability of the rations. The amount of oil meal included in each ration was determined by the protein content of the meal and the desired level of ration protein, 4, 6, or 8%. A mixture of equal parts of starch and glucose was added to bring the total to 600 gm. The chemical composition of the rations is given in table 1.

The experimental animals were 24 lambs weighing from 55 to 75 lb. For convenience in conducting digestion and nitrogen balance trials, the lambs were handled in two groups of 12 each. A total of six trials, two at each level of protein intake, were conducted. In each trial, which involved a group of twelve lambs and three rations, four lambs were assigned at random to the same ration with respect to oil-meal supplement. The

[1] Departments of Animal Husbandry and Biochemistry.

4% protein rations were compared in the first two trials, the 6% rations in the next two trials and the 8% rations in the last two trials. Each trial consisted of a 7-day collection period preceded by a 10-day preliminary period. During both periods the lambs were fed a constant daily amount (600 gm.) of ration in two equal portions. Feces and urine were

TABLE 1. PERCENTAGE COMPOSITION OF SELECTED FEEDS AND RATIONS (DRY MATTER BASIS)

Feeds and rations	Organic matter	Protein (N × 6.25)	Ether extract	Crude fiber	N-free extract
Feeds					
Soybean oil meal	93.0	51.1	1.4	5.3	35.2
Sesame oil meal	87.8	49.6	7.2	5.0	26.0
Cottonseed meal	93.5	43.1	4.0	13.6	32.8
Cellulose (Solka floc)	100.0	——	——	84.0	16.0
Rations					
4% protein:					
Soybean oil meal	94.9	4.4	6.0	30.3	54.2
Sesame oil meal	94.5	4.4	6.5	30.2	53.4
Cottonseed meal	95.0	4.4	6.1	31.0	53.5
6% protein:					
Soybean oil meal	94.6	6.5	6.1	30.5	51.5
Sesame oil meal	94.0	6.3	6.6	30.3	50.8
Cottonseed meal	94.4	6.5	6.4	31.7	49.8
8% protein:					
Soybean oil meal	94.4	9.0	6.0	30.6	48.8
Sesame oil meal	93.6	8.3	7.0	30.5	47.8
Cottonseed meal	94.4	8.5	6.9	32.1	46.9

collected and sampled as described by Tillman and Swift (1953). Lambs that refused feed were taken off experiment. At the end of each collection period the lambs were removed from the metabolism crates (Briggs and Gallup, 1949) and during a 10-day standardization period preceding the next trial they were fed a 9% protein ration.

Chemical analyses were made on feeds and excreta according to standard procedures (A.O.A.C., 1950). Analysis of variance was conducted as described by Snedecor (1946).

Results and Discussion

The average digestibility of nutrients in the different rations together with nitrogen balance data are shown in table 2.

With but few exceptions, there was a progressive increase in the digestibility of various nutrients as the level of protein in the three oil meal rations was increased from 4% to 6% and 8%. This effect of level

TABLE 2. DIGESTIBILITY AND NITROGEN BALANCE DATA OBTAINED WITH LAMBS FED DIFFERENCE PROTEIN SUPPLEMENTS AT DIFFERENT LEVELS

Items compared	4% Protein ration			6% Protein ration			8% Protein ration		
	Soybean oil meal	Sesame oil meal	Cottonseed meal	Soybean oil meal	Sesame oil meal	Cottonseed meal	Soybean oil meal	Sesame oil meal	Cottonseed meal
No. of lambs	7	7	6	8	6	5	8	7	8
Dry matter intake, gm.	558.8	559.4	560.4	561.3	563.2	560.0	560.3	561.2	561.0
Digestibility, %									
Organic matter	60.1	58.5	62.4	71.4	68.1	62.1	78.3	76.1	74.0
Protein	27.3	29.9	20.8	38.7	40.1	32.3	58.3	52.4	43.7
Ether extract	85.7	89.8	87.2	88.1	86.3	91.7	88.7	92.8	93.1
Crude fiber	29.4	24.5	33.5	59.5	52.2	41.4	72.8	70.0	68.8
N-free extract	77.1	76.3	76.1	80.5	78.6	75.4	84.0	81.7	80.4
Nitrogen balance, gm.									
Nitrogen intake	3.95	3.96	3.91	5.82	5.67	5.82	8.10	7.47	7.61
Nitrogen in feces	2.85	2.77	3.09	3.56	3.39	3.93	3.38	3.56	4.29
Nitrogen in urine	1.88	1.82	1.71	2.45	2.27	2.18	3.44	2.48	2.15
Nitrogen retention	—0.81	—0.63	—0.89	—0.20	0.01	—0.29	1.28	1.44	1.18
Percent nitrogen retained	—20.5	—15.9	—22.8	—3.4	0.2	—5.0	15.8	19.3	15.5

of protein on digestibility was made especially evident by the increase in digestibility of crude fiber from about 30% in the 4% protein rations to 70% in the 8% rations. Increases in the digestibility of other nutrients, except protein, itself, were less marked.

At each of the three protein levels, the apparent digestibility of protein was significantly lower ($P<0.01$) in the ration containing cottonseed meal than in those containing either soybean or sesame oil meals. The magnitude of these differences ranged from 6 to 14 percentage units. Other differences in digestibility were small and failed to establish a trend related to oil meal supplement. There was a significant ($P<0.01$) interaction between level of protein and source, the digestibility of protein being lower in the soybean oil meal ration than in the sesame oil meal ration at the 4% and 6% levels but higher at the 8% level. At the latter level, however, the higher percentage of protein in the soybean oil meal ration, 9% as compared to 8.3%, was an influential factor.

The lambs were in negative nitrogen balance on the 4% protein rations, in nitrogen equilibrium or close thereto on the 6% rations and in positive nitrogen balance on the 8% rations. At each of these protein levels average daily nitrogen retention expressed in grams or as a percentage of the intake was lowest for the lambs on the cottonseed meal rations and highest for those on the sesame oil meal rations. High fecal nitrogen excretion by lambs on the cottonseed meal rations accounted largely for these differences, urinary nitrogen excretion on the cottonseed meal rations being reduced but not sufficiently to compensate for the fecal nitrogen increase. Regression analysis of nitrogen retention on nitrogen intake failed to show statistically significant differences between rations; nevertheless, the results show a trend that is in substantial agreement with the results of a previous study (Woods *et al.*, 1957) which demonstrated a difference in protein value between rations containing cottonseed meal and those containing either sesame meal or a combination of sesame and soybean oil meals. They are in agreement also with the results of Briggs *et al.* (1956) with respect to differences between cottonseed meal and soybean oil meal in nitrogen digestibility. The relatively high crude fiber content of cottonseed meal, 13% as compared to 5% in soybean oil meal in the present experiment, and the presence of hulls which contain indigestible protein are factors probably contributing to low apparent digestibility of total nitrogen in the commercial meal.

Summary

In digestion and nitrogen balance trials with lambs, soybean oil meal, sesame oil meal and cottonseed meal were compared as protein supplements in semi-purified rations containing 4, 6 and 8% protein.

The digestibility of protein at each protein level was significantly lower in the cottonseed meal ration ($P<0.01$) than in either of the other

two rations. Also, average nitrogen retention at each protein level was lowest with the cottonseed meal ration and highest with the sesame oil meal ration. Although these differences in retention were not statistically significant, the trend of results was in accord with previous observations with lambs fed 9% protein rations containing sesame oil meal and cottonseed meal as protein supplements.

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COMPARISON OF LAMBS FROM WESTERN (COLUMBIA-RAMBOUILLET) EWES AND SIRED BY RAMS OF FOUR DOWN BREEDS [1]

W. E. NEVILLE, JR.,[2] A. B. CHAPMAN AND A. L. POPE [3]

University of Wisconsin, Madison

MANY fat lambs produced for early slaughter in lamb producing areas of the United States result from mating Western type ewes with various breeds of Down rams. The results from various crossbred matings have been reported for certain birth characteristics of lambs and other farm animals (Miller, 1935; Jamison *et al.*, 1956; Moore *et al.*, 1956; Walton and Hammond, 1938) and for weaning characteristics of lambs under range conditions (Hultz *et al.*, 1935; Neale, 1943) and West Coast conditions (Miller, 1935; Nelson *et al.*, 1950; de Baca *et al.*, 1956) but only to a limited extent under Mid-West (Miller and Dailey, 1951) and Southern conditions (Carter *et al.*, 1957).

The objectives of Experiment I (birth characters) were to determine whether or not lambs sired by Suffolk, Hampshire and Shropshire rams and out of Western (Columbia-Rambouillet) ewes differed in birth weight and in measurement of body parts which might be associated with difficult parturition.

The objectives of Experiment II (120-day characters) were to determine under Wisconsin farm conditions (1) whether there were indications of real differences in economically important characters between first-cross lambs from Western ewes sired by different breeds of Down rams, (2) the extent to which these traits were affected by sex and type of birth of the lamb, and (3) the degree of phenotypic association among these traits.

Materials

In Experiment I all the ewes were obtained from Montana and were largely of Rambouillet and Columbia breeding. The 30 rams used in this study of birth characters as well as the 40 rams used in Experiment II were believed to be representative of their respective breeds in Wisconsin.

Birth Characters. Data for Experiment I were collected during 1954 and 1955 at the University of Wisconsin. The rams used consisted of 10

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[2] Present address: Georgia Experiment Station, Experiment, Georgia.

[3] Credit is given to Dr. Newton Morton, Genetics Department, University of Wisconsin, for suggesting the method of statistical analysis which was used in this study. Also gratefully acknowledged is the cooperation and help of the staff of Oscar Mayer and Company in making the carcass phase of this study possible.

rams each of Suffolk, Hampshire and Shropshire breeds. All the ewes in 1954 (28 in number) were well-developed two-year-olds at lambing. The ewes in 1955 included most of those used the previous year in addition to a small number of aged ewes, making a total of 72 ewe and 92 cross-bred lamb observations for both years. The assignment of ewes to rams was at random.

Data collected on the ewes included width of pins (distance between the outer edges of the tuber ischii), width of hips (distance between the outer edges of the tuber coxae) and notations of observed deviations from the "normal" birth presentation, e.g., head turned back, etc. Also noted were whether or not the ewe required assistance during parturition and whether or not birth was considered normal.

Data collected on the lambs included birth weight, head width (widest point between the outer edges of the supraorbital process), head length (distance between the dorsal part of the occipital bone and end of nostril) and circumference of the body at the shoulder and chine. These latter two measurements were taken with a small steel tape while the lamb was held over a small barrel to simulate normal birth position.

The 120-Day Characters. Data for Experiment II were obtained from Wisconsin flocks whose owners agreed to cooperate with the University in a two-year crossbreeding study. A combination of two or more breeds of rams from the Suffolk, Hampshire, Oxford and Shropshire breeds were used on Western ewes. Records from nine farms were included in the study both years while seven farms were included for one year only; four in 1955 and three in 1956.

On each farm, the ewes ran together continuously except during the fall when they were randomly assigned to breeding groups. There were at least two and not more than four of these ewe groups per farm with a different breed of ram in each group.

When the lambs on each farm averaged 120 days of age, the senior author recorded the body weight, staple length on the side and market grade on each lamb except those considered to be outlying observations due to wide deviation in birth date. Corrections were made for weight and staple length of lambs whose ages differed from 120 days at the time these data were collected. The correction method used has been described by Phillips and Brier (1940). Market grade was determined by the covering over the ribs and back. Data from 357 Suffolk, 366 Hampshire, 475 Oxford and 343 Shropshire sired lambs were used in the analysis of these three 120-day characters.

Two ewe and two wether lambs from each sire were purchased for carcass studies. They were selected at random within type of birth and narrow age limits. The lambs were brought to a Madison packing plant for slaughter. Carcass data collected included hot carcass weight, Federal carcass grade, circumference of both thighs at the widest point, cross-sectional length and area of the *longissimus dorsi* from a cut made between

the 12th and 13th ribs, and height of spinous process (distance between dorsal part of the spinal canal to the tip of the process) at the 13th rib.

Method of Analysis

The type of analysis used is an extension of the R×2 analysis for disproportionate subclass numbers (Snedecor, 1948) to a $2^n \times R \times C$ type of analysis. This extension was used to allow for fitting constants as described by Snedecor (1948). Breed, sex and type of birth were considered fixed effects while year and farm were considered random effects. Proper error terms for the various models which include fixed and random effects are clearly described by Schultz (1955). When the analysis revealed significant breed differences, Duncan's (1955) test was used to isolate breed differences contributing to the significance.

In the case of breed, sex and type of birth effects for birth characters, a large "F" value was required for significance since the degrees of freedom were two and two for breed and one and one for sex and type of birth. Parametrically, the interaction mean square should be at least as large as the individual mean square. In 12 of 15 cases, this interaction statistic was slightly smaller than the sample individual mean square. Since only 68 degrees of freedom were used in obtaining the interaction mean square, the individual mean square should be a more precise value than the interaction mean square for tests of significance and hence was used as the error term.

Results

Birth Characters. Thirteen percent of the ewes required assistance at lambing. Chi-square analysis did not show significant breed of sire or year differences in the number of ewes which required assistance. The three mating groups of ewes were not found to be significantly different in the width of their hips or pins at parturition. Interaction of year with ewe was not significant, but the analysis did indicate that the ewes were significantly wider at the pins in 1955 than in 1954.

In general, breed of sire and type of birth effects on lambs at birth were significant while sex and all the interactions were not. Year effects were significant only for the two circumference measurements. This could have been due to a change in personnel taking the measurements or to age of dams. Table 1 gives the weighted means, breed constants and differences in sexes, types of birth and years for the five characters studied at birth. Suffolk sired lambs were significantly heavier and larger than Shropshire sired lambs in all measurements except head width. The differences between the Suffolk and Hampshire sired lambs were not significant for any of the five characters at birth.

Breed of sire means for each character were adjusted by covariance for differences in birth weight and the data were reanalysed. On this basis,

Hampshire-sired lambs had significantly wider heads, than Suffolk or Shropshire and significantly longer heads than Shropshire-sired lambs. Breed of sire differences in other weight-adjusted birth characters were not significant.

The 120-Day Characters. The results of tests of significance for main effects and their interactions for live weight, staple length and live grade at 120 days of age indicate that breed, farm, sex and type of birth were significant while their interactions were not significant sources of variation.

TABLE 1. WEIGHTED MEANS, BREED CONSTANTS, SEX, TYPE OF BIRTH AND YEAR DIFFERENCES [a] FOR FIVE BIRTH CHARACTERS OF WESTERN CROSSBRED LAMBS

Character	Weighted mean (u)	Breed constants [b]			Male minus female	Single minus twin	1955 minus 1954
Birth weight, lb.	11.08	Shropshire −.98	Hampshire .08	Suffolk .90	.72*	1.96**	.30
Head width, cm.	7.38	Shropshire −.28	Suffolk .08	Hampshire .21	.16	.24	.20
Head length, cm.	12.47	Shropshire −.46	Hampshire .17	Suffolk .29	.32	.62**	.12
Circumference at shoulder, cm.	34.45	Shropshire −1.10	Hampshire .03	Suffolk 1.07	.82	2.72**	3.22**
Circumference at chine, cm.	36.33	Shropshire −1.22	Hampshire .21	Suffolk 1.01	−.004	2.48**	3.32**

[a] Numerical sum, ($\Sigma|i|$), of the two component constants.
[b] Any two constants not underscored by the same line are significantly different.
* $p<.05$.
** $p<.01$.

Weighted means, breed constants and sex and type of birth differences by years for these characters are presented in table 2. The 120-day weights of the Hampshire and Suffolk sired lambs were significantly heavier than the Shropshire sired lambs in both years. Also, for both years, the Suffolk lambs were significantly heavier than the Oxford lambs. Other breed comparisons were inconsistent in significance from one year to the next. The best estimate (weighted averages) of the extent to which wethers were heavier than ewes and singles were heavier than twins was 2.79 and 9.64 lb., respectively.

For both years the Suffolk lambs had significantly shorter staple length, than the Oxfords. Ewes and singles had longer staple (weighted averages) by 0.23 and 0.18 centimeters than wethers and twins, respectively.

The largest significant difference in live grade was that of Hampshire lambs grading 0.57 of a grade higher than Oxford lambs in 1955. In 1956, however, the difference between these two breed groups of lambs was only 0.24 of a grade and not significant. Hence, population differences between these breed groups are not clear-cut in view of significant breed-year interactions. Singles graded significantly higher than twins both years while the difference between sexes in this character was significant the first year only.

The weighted means, breed constants and the sex differences by years for the unadjusted carcass characters are given in table 3. Results of analyses of these data show that breed effects were not significant but that sex, farm effects and farm-breed interactions were important sources of variation in almost all traits. Carcass weight adjustment of the other five carcass traits reduced the number of significant farm-breed interactions. The adjustment produced virtually no change in the significance of farm and breed effects except in the case of carcass grade for 1955. That year,

TABLE 2. WEIGHTED MEANS, BREED CONSTANTS, SEX AND TYPE OF BIRTH DIFFERENCES[a] GIVEN BY YEARS FOR THREE 120-DAY CHARACTERS OF WESTERN CROSSBRED LAMBS

Character	Year	Weighted mean (u)	Breed constants[b]				Wether minus ewe	Single minus twin
120-day weight, lb.	1955	67.02	Shropshire −3.47	Oxford −2.41	Hampshire .76	Suffolk 5.13	3.14**	10.44**
	1956	66.58	Shropshire −3.96	Oxford −.32	Hampshire 1.42	Suffolk 2.85	2.46	8.90**
120-day staple length, cm.	1955	3.85	Suffolk −.30	Hampshire −.13	Shropshire .17	Oxford .27	−.20**	.10
	1956	3.54	Suffolk −.31	Hampshire .03	Shropshire .07	Oxford .22	−.28**	.26**
120-day live grade[c]	1955	2.77	Hampshire −.21	Shropshire −.11	Suffolk −.04	Oxford .36	.12*	−.56**
	1956	2.85	Hampshire −.13	Suffolk −.13	Oxford .11	Shropshire .28	.06	−.48**

[a] Numerical sum, (Σlil), of the two component constants.
[b] Any two constants not underscored by the same line are significantly different.
[c] Prime=1, choice=2, good=3, utility=4, and cull=5.
* $p<.05$
** $p<.01$.

Hampshire and Shropshire graded better than Oxford and Suffolk. The adjustment also caused a reduction of one-half to three-fourths in the range of the unadjusted farm and breed effects.

TABLE 3. WEIGHTED MEANS, BREED CONSTANTS AND SEX DIFFERENCES [a] BY YEARS FOR CARCASS CHARACTERISTICS OF WESTERN CROSSBRED LAMBS [b]

Character	Year	Weighted mean (u)	Breed constants [c]				Wether minus ewe
Carcass weight, lb.	1955	37.71	Shropshire −2.03	Oxford −1.32	Hampshire −.01	Suffolk 3.36	1.46
	1956	34.90	Oxford −1.40	Shropshire −.94	Hampshire −.40	Suffolk 2.74	1.74
Cross-sectional length of longissimus dorsi, cm.	1955	5.27	Oxford −.27	Shropshire −.03	Hampshire .02	Suffolk .29	.22*
	1956	5.41	Shropshire −.15	Oxford −.03	Hampshire .05	Suffolk .14	.20*
Cross-sectional area of longissimus dorsi, sq. cm.	1955	9.74	Oxford −.54	Shropshire −.28	Hampshire .20	Suffolk .62	.82**
	1956	10.36	Shropshire −.54	Hampshire −.40	Suffolk .42	Oxford .52	.42
Height of spinous process of vertebra, cm.	1955	2.19	Oxford −.15	Shropshire .00	Suffolk .07	Hampshire .08	.04
	1956	2.26	Shropshire −.05	Hampshire −.02	Oxford .01	Suffolk .06	.08
Circumference of thighs, in.	1955	21.48	Shropshire −.56	Oxford −.09	Hampshire −.04	Suffolk .69	.44**
	1956	21.99	Shropshire −.35	Oxford −.20	Hampshire −.04	Suffolk .59	.12
Carcass grade [d]	1955	3.14	Hampshire −.25	Shropshire −.07	Suffolk .06	Oxford .26	.24*
	1956	2.64	Suffolk −.09	Shropshire −.07	Hampshire −.02	Oxford .18	−.02

[a] Numerical sum, (Σli), of the two component constants.
[b] The values for the five traits (other than carcass weight) are unadjusted for carcass weight in this table.
[c] Any two constants not underscored by the same line are significantly different.
[d] Prime=1, choice=2, good=3, utility=4 and cull=5.
* $p<.05$.
** $p<.01$.

Discussion

Birth Characters. In this study as well as that of Miller (1935) the breed of sire was found to produce significant differences among lambs at birth in weight and in measurement of certain body parts. These differences, however, were not found to be associated with parturition difficulty (dystocia). The ewes in the present study, like those of Miller, were well-developed. Jamison *et al.* (1956) also found breed of sire differences in size of lambs at birth, but did not report on the relationship between breed of sire and dystocia.

Walton and Hammond (1938) reported that three Shetland mares had no trouble giving birth to Shire crossbred foals. It is assumed that the Shetland mares were mature since they were "average size" (200 kilograms) for Shetlands. For uniparous animals, these results suggest that mature females are capable of regulating fetus size to a point where the fetus can usually pass through the dam's birth canal without complication.

The negative results found in the present study do not mean that dystocia would not be found with smaller, younger or poorly developed ewes. In beef cattle, Moore *et al.* (1956) found that the size of the sire did contribute to dystocia among immature heifers. They also found that heifers experienced less difficulty with female calves whose weight was, on the average, about 4 to 5 lb. less than the weight of males at birth.

Dystocia is often associated with abnormal birth position. The present study did not show any influence of breed of sire on birth position nor were any research reports on this kind of effect found.

The 120-Day Weight. The differences in 120-day weights of lambs in this study are very similar to those obtained by Miller (1935) for the same breeds of sire. In both studies, Suffolk and Hampshire sired lambs weighed significantly more than those sired by Shropshires.

In Miller's work, which was done under good conditions for fat lamb production, the lambs sired by Down rams were 3.0 to 8.0 lb. heavier than those sired by the fine wool rams. Under range conditions, however, Hultz *et al.* (1935) and Neale (1943) found the Down-Rambouillet lambs weighed no more at weaning than straightbred Rambouillet lambs. Neale (1943) reported that under poor range conditions lambs of the latter group weighed more than those of the former. Under Virginia conditions, Carter *et al.* (1957) found that Down-sired lambs from ewes of Suffolk ×Rambouillet and Hampshire×Rambouillet breeding gained faster than Down-sired lambs from grade Rambouillet ewes.

In the present study, the 9.6- and 2.8-lb. advantage of singles over twins and wethers over ewes, respectively, is in good agreement with the respective 10.0- and 3.5-lb. advantage obtained by averaging the reported results of fine wool ewes bred to Down rams.

In the main part of the present study, no straightbred lambs of either Western or Down breeds were used. However, a comparison of the 120-day lamb weights of the crossbreds in the main part of the study with

those of straightbred Down lambs was available from the records of the Wisconsin Sheep Improvement Program. There were recorded 1,892 Suffolk, Hampshire and Shropshire lambs in 1955 and 1956. Average 120-day weights were obtained for each farm within breed, year, sex and type of birth for straightbred and first-cross Down-Western lambs of Suffolk, Hampshire and Shropshire breeding. Unweighted means were then calculated for each breed within mating system. These were 75.1, 68.2 and 61.8 lb. for the straightbred Suffolk, Hampshire and Shropshire, respectively and 68.5, 65.3 and 62.3 lb. for the comparable crossbreds.

Under the assumption that the 120-day weights resulting in the crossbreds are due to additive gene effects, three independent estimates of 120-day weights of straightbred Westerns can be made. Twice the crossbred average minus the straightbred Down breed average should equal the average weight of the straightbred Western. These estimates of the straightbred Western 120-day weights obtained from Suffolk, Hampshire and Shropshire data were 61.9, 62.4 and 62.8 lb., respectively. The three independent additive estimates of straightbred Western weights are in good agreement. The similarity between the three predicted results, not their absolute values, would also be consistent with a constant heterotic effect from one Down-Western combination to another. No basis for distinguishing between these two alternatives is evident in these data.

The 120-Day Staple Length. Staple length is not an important characteristic in fat lamb production. In the selection of ewe lambs for flock replacements, however, the significantly longer staple length of the Oxfords over the Suffolks would be of some economic importance.

Carcass Characters. Since just two wether and two ewe lambs per sire were chosen for carcass studies only very large differences in carcass traits would be expected to be significant.

Walker and McMeekan (1944) and Palsson (1939) reported correlations of 0.74 and 0.67, respectively, between the cross-sectional length of the *longissimus dorsi* and the total weight of the muscle (obtained by dissection) in the carcass. The former workers, however, obtained a smaller correlation ($r=0.19$) when approximate muscle area was used as a variable instead of muscle length. Since none of the carcasses in this study were dissected, only indirect associations of breed differences in muscle weight were available. The present analysis indicated that breeds did not differ significantly in the unadjusted or weight-adjusted data for cross-sectional area of this muscle. Significant breed differences were present in one year for cross-sectional length in the unadjusted data and increased in significance in the weight-adjusted data. However, on the basis of both years' results, population differences between breed groups for this character are not clear-cut.

Width of carcass at thighs was a measurement of the whole carcass that Walker and McMeekan (1944) found to be correlated with percent lean in the carcass ($r=0.67$). A similar measurement, circumference of

thigh, was used in this study. Although there were no significant breed differences for this character, the breeds occupied the same position in the array both years in the unadjusted and adjusted data with the exception of an interchange between the Hampshires and Oxfords in the adjusted data for 1955.

In view of the previously reported carcass associations found by Palsson (1939) and by Walker and McMeekan (1944) three correlations were calculated on weight-adjusted data within year, breed, farm and sex. Correlations of 0.25, 0.09 and 0.12 were obtained when length of the *longissimus dorsi* was correlated with (1) circumference of the thighs (2) height of spinous process of the vertebra and (3) carcass grade, respectively.

Differences between the breeds in the way they "handle" over the back and loin have led some stockmen to believe that some breeds (e.g., Oxford) have higher spinous processes of the vertebrae than that of other breeds (e.g., Shropshire). However, this study failed to reveal significant breed or sex differences in this characteristic.

The only evidence of significant breed differences in carcass grade was in 1955 in the weight adjusted data. Hampshires and Shropshires graded significantly higher than Oxfords and Suffolks. It is important to bear in mind, however, that statistical weight adjustment may not be a realistic reflection of what can happen in nature. A within year, breed, farm, sex and type of birth correlation of $-.55$ ($P<.01$) was obtained between carcass weight and carcass grade. Hence, there was a tendency for the heavier carcasses to grade better than the lighter ones.

Summary

Breed of sire differences among crossbred lambs at birth were determined from a two-year study. The lambs were first-crosses resulting from mating Suffolk, Hampshire, and Shropshire rams to Western ewes. The characters studied on the lambs at birth were weight, head width, head length and circumference at chine and shoulder points. The characters studied on the dams at parturition were width at hips, width at pins and parturition difficulty which was believed to be due to shape, size or position of the fetus.

Breed of sire differences among crossbred and straightbred lambs at 120 days of age were determined from a two-year collection of data from Wisconsin farms. The crossbred lambs were first-crosses from Western ewes bred to Suffolk, Hampshire, Oxford and Shropshire rams. The 120-day characters studied were live weight, staple length and live grade. The straightbred lambs (purebreds and high grades) were of Suffolk, Hampshire and Shropshire breeds. Four crossbred lambs, two females and two wethers, from the progeny of each sire were used for carcass studies. The carcass characters studied included carcass weight, cross-

sectional length and area of the *longissimus dorsi*, height of the spinous process of the vertebra, circumference of both thighs and U.S. carcass grade.

Shropshire sired crossbred lambs were significantly less in birth weight and in the sizes of various body parts than Hampshire and Suffolk sired lambs. The last two did not differ significantly from each other in any of the birth characters. These differences gave no evidence of being associated with dystocia.

Fitting constants to the data gave 120-day weights for the Suffolk, Hampshire, Oxford and Shropshire sired crossbred lambs of 70.8, 67.9, 65.4 and 63.1 lb., respectively. Suffolk and Hampshire crosses weighed significantly more than Shropshire crosses and Suffolks than Oxfords in each year. The other breed differences in 120-day weight were consistent in direction over both years but not significant in both years. Wethers and singles weighed significantly more than females and twins by 2.8 and 9.6 lb., respectively.

The weights of straightbred Down breed lambs (Suffolk, Hampshire and Shropshire) were found to rank in the same order as the crossbreds by the same breed of sire. There was a high degree of consistency from group to group in favor of the straightbreds for Suffolks and Hampshires and in favor of the crossbreds for the Shropshires when compared within sex, type of birth and year groups. Three independent additive estimates of the 120-day weights of the Westerns based on the comparison of the Suffolk, Hampshire and Shropshire straightbreds with their Western crossbreds were highly consistent. This implies that the combinability of Westerns with the Down breeds for 120-day weights is genetically simply additive or reflects a constant heterotic effect.

For live grade, there were no breed differences which were consistently significant from one year to the next. Singles graded a significant 0.5 of a grade higher than twins. Carcass characters were analyzed on the basis of carcasses unadjusted as well as adjusted for weight. Regardless of the adjustment, there was little evidence of breed differences for any of the carcass characters.

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THE EFFECT OF STARVATION AND SUBSEQUENT REFEEDING ON SOME ACTIVITIES OF RUMEN MICROORGANISMS *IN VITRO*[1]

J. C. MEISKE,[2] R. L. SALSBURY, J. A. HOEFER AND R. W. LUECKE[3]

Michigan State University, East Lansing

FEEDER cattle are frequently shipped long distances by rail, which means that they go without feed or water for a considerable period of time. The practical livestock man tries to take the animal through an adjustment period by gradually bringing it up to full feed. Fasting or starvation is known to influence the microbial population of the rumen, but to what extent this affects the animal's ability to respond to feed is not known.

The experiments reported herein are preliminary studies on the effects of periods of starvation on the return of microorganisms to a normal rate of certain metabolic activities. The ability of rumen microorganisms to digest cellulose *in vitro* and to lower the viscosity of a stable suspension of a cellulose derivative, carboxymethylcellulose[4] (CMC), was used as a measure of their activities in samples taken at different times. The effect of using different roughages before and after starvation was also studied.

Experimental

Sampling. The inocula for the laboratory studies were obtained from a four-year-old Hereford steer fitted with a permanent rumen fistula. Samples of the fluid portion of the ingesta were strained through a single layer of cheesecloth to remove the larger plant particles. The strained fluid was collected in a thermos flask that had been warmed with water and thoroughly gassed with carbon dioxide to produce anaerobic conditions. The pH of the rumen fluid was determined as soon as the samples were brought to the laboratory. Limited microscopic observations were made on the strained rumen fluid each time.

In vitro Cellulose Digestion. The fermentation procedure used was a modification of the method described by Huhtanen *et al.* (1954) and was like that of Salsbury *et al.* (1956). The incubation periods were 3, 6, 9 and 24 hours. The samples were all run in triplicate. However, only the 24-hour fermentation period results are reported here as they best show the differences in sample activities at different times of sampling. The

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 2203.

[2] Present address: Department of Animal Husbandry, University of Minnesota, St. Paul.

[3] Departments of Agricultural Chemistry and Animal Husbandry.

[4] The sodium salt. CMC-50, Hercules Powder Company, Wilmington, Del.

method of Crampton and Maynard (1938) with modifications (Salsbury *et al.*, 1956) was used for determination of cellulose.

Viscosimetric Studies. It has been shown that there are microorganisms present in the rumen which produce enzymes capable of attacking the cellulose derivative carboxymethylcellulose (Underkofler *et al.*, 1953; Kitts and Underkofler, 1954; King, 1956). Changes in viscosity of CMC suspensions have been used to demonstrate production of these enzymes (Levinson and Reese, 1950; Reese *et al.*, 1950; Jermyn, 1952; King, 1956; Gill and King, 1957). The method used here involved measuring the change in viscosity of CMC brought about by rumen fluid fractions. Viscosity was measured by determining the time required for a definite volume of the CMC solution to flow through a calibrated capillary.[5]

The substrate used consisted of a 0.3% solution of CMC. About 400 ml. of water were heated to 80° C. and added, together with 1.5 gm. of CMC, to a Waring blendor. These were mixed in the blendor of 25 minutes, transferred to a 500 ml. volumetric flask, cooled and brought to volume.

Eight ml. of 0.3% CMC substrate, 1 ml. of pH 6.9 phosphate buffer (ionic strength = 0.145), and 1 ml. of the rumen fluid fraction to be tested, were placed in a viscosimeter in that order. The viscosimeters were suspended in a constant temperature water bath maintained at 37° C. The efflux time of the test solutions was divided by the efflux time for distilled water to give a relative viscosity value. Densities of water and rumen fluid were not sufficiently different to appreciably affect results and, hence, were not considered in the calculations. The zero-time viscosity for test solutions was that obtained from a solution containing rumen fluid that had been boiled to inactivate the enzymes. Subsequent readings were taken at 10, 20, 30, 40 and 60 minutes after introduction of the unheated sample. However, it was decided that the difference between initial and 10-minute viscosity readings gave the best measure of the activity of the samples used.

Data on two fractions were recorded. One fraction consisted of the strained material. The other fraction (hereafter called S-1) was prepared by centrifuging the strained rumen fluid at 1500 r.p.m. for 5 minutes in an International size 2 centrifuge equipped with a four-place head. This removed the protozoa and plant material from the inocula. The S-1 was a cloudy yellowish fluid.

During the last two trials, and for the last sample from the first trial, a third fraction of rumen fluid was tested. This fraction (hereafter called S-2) was prepared by centrifuging the S-1 fraction at 12,000 r.p.m. for 110 minutes in a refrigerated Servall centrifuge (SS-1 head). This operation removed the bacteria from the S-1 and resulted in a cell-free preparation of rumen fluid. The S-2 was a clear yellow solution.

Experimental Rations. During all feeding periods the steer was fed twice

[5] Ostwald-Cannon-Fenske viscosimeters, size 200, were used. The efflux times for distilled water at 37° C. ranged from 8.3 to 9.7 seconds.

daily a ration of 10 lb. of hay and 1 lb. of supplement. The composition of the supplement is given in table 1.

Two preliminary trials with 48-hour starvation periods produced results similar to the trials reported here, though differences were not as pronounced. In the trials reported here, the steer was starved for 72 hours. Water was available at all times during the trials.

The zero-hour sample was obtained just prior to the morning feeding or about 15 hours after the last feeding.

During Trial I, the steer received timothy hay before and after the starvation period; during Trial II, timothy hay before and alfalfa hay after; during Trial III, alfalfa hay before and after; and during Trial IV, alfalfa hay before and timothy hay after starvation.

TABLE 1. COMPOSITION OF THE STEER SUPPLEMENT

Ingredients	Percent of mixture
Corn cobs (ground)	30.0
Soybean oil meal (solvent extracted)	32.25
Dried molasses	10.0
Alfalfa meal (dehydrated)	15.0
Dicalcium phosphate	5.0
Trace minerals	0.2
Vitamin A and D concentrate	0.05
Trace mineralized salt	1.5
Urea	6.0

Results

Microscopic observations made during each trial showed that similar changes occurred in each experimental period. After the animal had been starved for 24 hours, the protozoa were less active and bacterial numbers were decreased. When feed had been withheld for 48 hours, the samples contained very few holotrich protozoa and few oligotrich protozoa. Occasional long chains of coccoid bacteria were noted. Some bacteria were in clumps. At the end of the 72-hour starvation period no holotrichs were ever noticed. Oligotrichs were few in number and quite inactive except in the last trial when they were quite active. The number of bacteria was noticeably decreased in these samples. What little plant material was present was usually covered with coccoid bacteria. In the first samples taken 3 hours after refeeding there were apparent increases in the number of bacteria. These increases were quite noticeable in the samples taken 24 hours after refeeding. Oligotrichs increased in number and activity as refeeding proceeded. Holotrichs, however, were apparently absent for about 5 days following a starvation period. In Trial III, 25 days had elapsed before a sample was observed to contain holotrichs. In this trial, the animal was given a rumen fluid transfusion of approximately 3 liters

from a normally fed steer on the thirty-second day of refeeding. All original forms of microbial life appeared to be present shortly thereafter.

Results of *in vitro* cellulose digestion are presented in table 2. In all trials the ability of rumen samples to digest cellulose *in vitro* was reduced 68 to 91% at the end of the 72-hour starvation period. In preliminary trials, starvation for 48 hours reduced the *in vitro* cellulose digestion only

TABLE 2. *IN VITRO* CELLULOSE DIGESTION IN ALL TRIALS

	Trial no.			
	I	II	III	IV
Sample time [a, b]	Cellulose digested			
	%	%	%	%
0	79.0	77.7	74.2	73.8
48–S	50.4	——	——	——
72–S	25.0	8.1	11.6	6.9
3–R	23.0	18.3	15.0	14.7
6–R	34.6	25.1	——	21.2
9–R	48.5	——	48.9	——
24–R	48.0	69.1	54.5	33.8
48–R	——	50.7	62.6	55.1
72–R	——	——	——	77.5
96–R	——	78.0	80.4	——
120–R	——	——	——	80.8
11D–R	——	——	81.0	——
19D–R	——	——	73.1	——

[a] Time elapsed from start of starvation or refeeding. Times are in hours unless otherwise indicated.
[b] Abbreviations: S=starvation, R=refeeding, D=days.

15 to 25%. It seems reasonable that the decline of *in vitro* cellulose digestion by rumen fluid obtained during starvation was due largely to a concomitant decline in the number of microorganisms per unit of rumen volume. *In vitro* digestion of cellulose was approximately normal within 3 to 4 days after refeeding. The pH of the rumen contents increased during the starvation periods from a normal value of about 6.8 to a maximum of 7.9. During the refeeding periods the pH dropped rapidly to values of 5.6 to 5.9 then gradually returned to the normal level.

Figures 1 and 2 show the changes in the viscosity of CMC suspensions brought about by rumen fluid fractions in Trials III and IV, respectively. The changes in viscosity observed in Trials I and II were quite similar to those shown in figure 2.

Discussion

Kitts and Underkofler (1954) suggested that cellulolytic enzymes of rumen microorganisms are adsorbed and carried on the cell surface. King

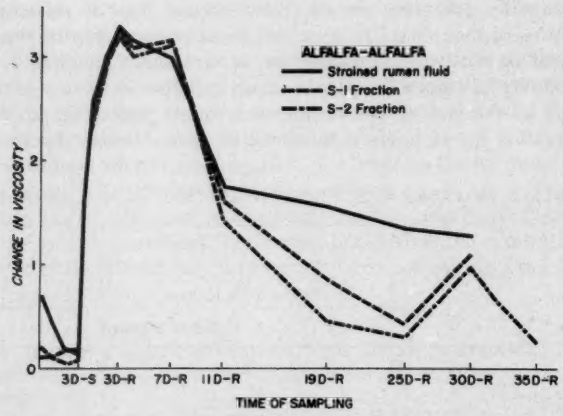

Figure 1. Change in viscosity of CMC by the different fractions of rumen fluid during Trial III.

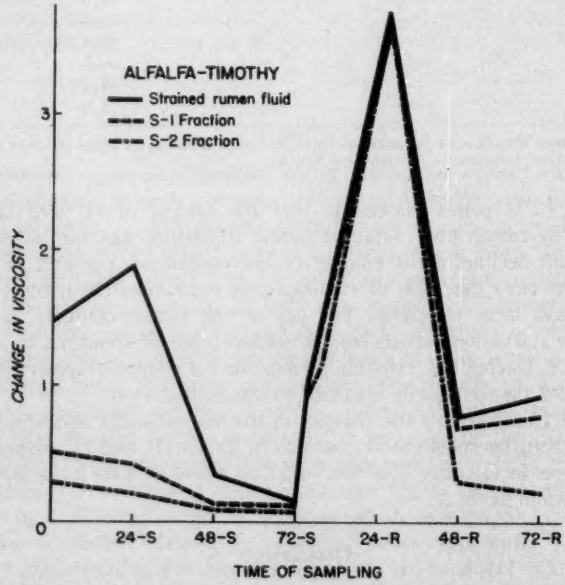

Figure 2. Change in viscosity of CMC by the different fractions of rumen fluid during Trial IV.

(1956), however, stated that a considerable portion of the cellulases are truly extra-cellular. According to the results presented in this paper, the S-2 fraction exhibited comparatively little activity when the steer was fed regularly. However, during early refeeding following starvation, the activities of all three fractions were approximately equal, indicating that the enzymes were then free of the cell. In all of these trials, all portions of rumen fluid obtained shortly after feeding had been resumed showed unusually high, but approximately equal, activity on CMC suspensions for at least a short time. A possible explanation is that the bacteria producing these enzymes may be faster growing than other rumen bacteria and that after the normal rumen population becomes re-established, they may be suppressed.

Another possible explanation lies in the report of King (1956) that many rumen samples contain a "cellulase inhibitor". If such an inhibitor were present in the experiments reported here, a reduction of its relative concentration by the starvation treatment might be expected to give fractions of higher activity.

The results reported here show that the ability to reduce the viscosity of CMC and the ability to hydrolyze cellulose are not necessarily related. This observation is in agreement with the suggestion of Halliwell (1957) that CMC and insoluble cellulose are not hydrolyzed by the same enzyme.

White *et al.* (1956) found that sheep starved for 9 days consumed an average of 460 g. of water per day. In the experiments reported here, the steer consumed no water during the starvation period after approximately the first 12 hours. The rumen contents decreased in volume and contained considerably less plant material as starvation progressed. Feces excreted during the last two days of starvation were noticeably drier than those excreted when the steer was fed regularly. The steer apparently experienced no alterations of appetite following a starvation period. Quin (1943) found that sheep starved for 65 hours required 3 days to attain their usual daily intake. Glucose-fermenting ability of these sheep returned to normal only after the sheep had been refed for 10 days. Coop (1949) reported that ingesta of a sheep starved 3 days hydrolyzed lotaustralin and fermented glucose normally after the sheep had been refed 12 to 24 hours. The data of Coop (1949), however, show that activity of samples at this time often surpassed the activity of samples taken when the sheep was regularly fed. White *et al.* (1956) found that after sheep had been starved 9 days they were consuming their usual amount of feed 2 or 3 days after feeding was resumed.

Summary

Samples of rumen fluid were collected at intervals from a fistulated steer fed (1) a high-roughage ration regularly, (2) no ration, starvation for 3

days, (3) refed (following starvation) a high-roughage ration. The abilities of rumen fluid to digest cellulose *in vitro* and to reduce the viscosity of a stable suspension of carboxymethylcellulose (CMC) were determined. A fraction (S-1) of rumen fluid freed of protozoa and most plant material and a second fraction (S-2) freed of bacteria, protozoa and plant material were also tested for activity on CMC.

The pH of rumen fluid rose continuously during the starvation period to a high between 7.65 and 7.94. The ability of rumen fluid to digest cellulose *in vitro* decreased greatly when the steer was starved for 3 days. All fractions of rumen fluid also decreased in their activity on CMC.

Fractions of rumen fluid obtained shortly after feeding had been resumed following starvation showed unusually high, but approximately equal, activity on CMC. The extremely high activities of the S-2 fractions indicated that the enzymes were largely extracellular at that time.

The ability to digest cellulose *in vitro* was normal in 3 to 4 days after feeding was resumed following starvation. Activities of the various fractions on CMC were normal when the steer had been refed 2 to 4 days after starvation with one exception. This exception might have been the result of a change in the normal proportion and metabolism of microbial life in the rumen.

No differences in the activities of rumen microorganisms could be attributed to the type of hay the steer received before or after the starvation period. The steer readily consumed all of its ration at each feeding.

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THE EFFECT OF CORN OIL UPON THE METABOLISM OF CALCIUM AND PHOSPHORUS BY SHEEP

ALLEN D. TILLMAN [1] AND JOHN R. BRETHOUR [2]

University of Tennessee—Atomic Energy Commission Agricultural Research Laboratory, Oak Ridge, Tennessee [3, 4, 5]

MANY workers (Swift *et al.*, 1951; Tillman *et al.*, 1954; Brooks *et al.*, 1954; Chappel *et al.*, 1955; Ward *et al.*, 1957; and Brethour *et al.*, 1958) have reported that the addition of corn oil to ruminant rations containing corn cobs or cottonseed hulls reduced the digestibility of all ration components except ether extract and that the detrimental effect was either completely or partially counteracted by alfalfa ash. In attempting to ascertain the element(s) in alfalfa ash responsible for this effect, Tefft (1954) found the "major" mineral fraction of "synthetic" alfalfa ash (Chappel *et al.*, 1955) effective in counteracting the detrimental effects of corn oil in semi-purified rations containing cottonseed hulls. Later Grainger *et al.* (1957) reported that the calcium in alfalfa ash was responsible for this beneficial effect. Thus, it seemed desirable to determine the effect of corn oil upon the metabolism of calcium and phosphorus by sheep.

Experimental Procedure

The composition of the basal (1) and corn oil (2) rations is shown in table 1. The daily feed for each animal consisted of 908 gm. of the designated rations.

Twelve wether lambs approximately 12 months old and weighing 75 to 80 lb. were randomly divided into two groups each of six animals. One group received the basal ration while the other group received the ration containing corn oil. Each animal received his ration in two equal portions twice daily during successive 14-day preliminary and 7-day collection periods. Daily collections of feces and urine were made while the sheep were in metabolism stalls described by Briggs and Gallup (1949) and modified by Hansard *et al.* (1951) for radioisotope procedures. At the beginning of the collection period, three animals from each group were given concurrently one millicurie each of calcium-45 and of phosphorus-32 by

[1] Research participant, Oak Ridge Institute of Nuclear Studies, on leave from Oklahoma State University.

[2] Present address, Kansas Agricultural Experiment Station, Hays.

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[4] The radioactive materials were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission. This study was completed under contract No. AT-40-1-Gen-242 between the University of Tennessee and the Atomic Energy Commission.

[5] The technical assistance of W. A. Lyke, B. Richmond and G. Tapp is acknowledged and sincerely appreciated.

intravenous injection, while the other three animals in each group were given the same dosage of each isotope orally. Calculations of fecal endogenous calcium and phosphorus were made by the isotope dilution method described by Kleiber *et al.* (1951), which was modified to allow a single injection of the isotope (Hansard, 1956), and by the comparative balance method described by Hansard (1952) and Comar *et al.* (1953). Blood samples, obtained from all animals by jugular puncture, were taken at frequent intervals during the collection period for the determination of

TABLE 1. PERCENTAGE COMPOSITION OF RATIONS

Ingredients	Ration 1	Ration 2
Cottonseed hulls	25.00	25.00
Alfalfa meal	15.00	15.00
Soybean oil meal	8.00	8.00
Corn, ground	40.00	40.00
Vitamins A and D [a]	0.10	0.10
Sodium chloride	0.50	0.50
Corn starch	5.45	1.70
Corn sugar	5.45	1.70
Dicalcium phosphate [b]	0.50	0.50
Corn oil	——	7.50

[a] Quadrex, courtesy Dr. M. Hockberg, NOPCO Chemical Co., Harrison, New Jersey, contained 20,000 I.U. of vitamin A and 2500 U.S.P. units of vitamin D_2 per gram.
[b] Reagent grade.

both calcium and phosphorus. Calcium was determined by the method of Clark and Collip (1925) and radiocalcium by a modified method described by Comar *et al.* (1951). Separation of radiocalcium from radiophosphorus was made by using an aluminium absorber having a mass of 55 mg/cm^2 to absorb the weaker beta emissions of radiocalcium. Details of the procedure and method used for self-absorption corrections in the case of radiocalicum were described by Comar *et al.* (1951). Inorganic phosphorus analyses were made by the method of Fiske and Subbarow (1925). Radioassay of both isotopes was performed by using a thin mica window Geiger-Mueller counter connected to a scaler. Statistical treatment of the data were by the analysis of variance method.

Results and Discussion

The results obtained in this trial are shown in table 2. The added fat did not affect significantly the utilization of dietary phosphorus, but it had a pronounced effect on dietary calcium. The apparent and true digestibilities as well as the retention of dietary calcium were reduced significantly ($P<0.01$) by the added fat. These results, obtained by a different technique, are in close agreement in regards to calcium with those of Grainger *et al.* (1957) and offer a possible explanation of the detrimental effects of

TABLE 2. THE EFFECT OF CORN OIL UPON CALCIUM AND PHOSPHORUS METABOLISM

Item	Ration 1, control	Ration 2, fat
Phosphorus		
Intake, gm.	2.905	2.905
Excretion, gm.	2.191	2.070
Feces	2.047	1.954
Urine	0.144	0.116
Fecal endogenous, gm.	1.495	1.217
Retained, % of intake	24.6	28.7
Apparent digestibility, %	29.6	32.7
Endogenous, % of intake	51.5	41.9
Endogenous, % of fecal	73.0	62.3
True digestibility	81.0	74.6
Calcium		
Intake, gm.	3.696	3.696
Excretion, gm.	2.913	3.287**
Feces, gm.	2.897	3.259**
Urine, gm.	0.016	0.028
Fecal endogenous, gm.	1.518	1.585
Retained, % of intake	21.2	11.1*
Apparent digestibility, %	21.6	11.8**
Endogenous, % of intake	41.1	42.9
Endogenous, % of fecal	52.4	48.6
True digestibility	62.7	54.7**

* Significant at the 5% probability level.
** Significant at the 1% probability level.

fat upon the utilization by ruminants of rations containing corn cobs or cottonseed hulls (Swift *et al.*, 1951; Tillman *et al.*, 1954; Ward *et al.*, 1957).

Fecal endogenous calcium excretion was not affected by the added fat, suggesting that the site of action of fat upon dietary calcium was within the gastrointestinal tract. In this connection, Calverley and Kennedy (1949) found that fats increased fecal calcium excretion in rats by the formation of indigestible calcium soaps, and that long-chain saturated fatty acids were more reactive than other fatty acids. Brooks *et al.* (1954) observed that the rumen ingesta obtained from sheep receiving a high level of corn oil or lard had a putrid odor and was very turbid. When alfalfa ash was added, the ingesta became clear and the odor disappeared, leading those workers to postulate that the minerals of alfalfa ash had aided in the emulsification of the added fat.

Many workers have reported that alfalfa ash counteracts either partially or completely the detrimental effects of fats in ruminant rations.

As this ash contains a mixture of mineral salts, the possibility that cations other than calcium might be made unavailable for absorption from the gastrointestinal tract cannot be disregarded. In fact, the results of Wooley and Mickelson (1954) point in that direction. When rabbits were fed a purified diet and different levels of fat, those workers found that added fat depressed gains and that this effect was counteracted by a mixture of minerals containing calcium, potassium and sodium salts, the sodium salt being more effective than either of the other salts. Brethour *et al.* (1958), however, reported that the addition of either potassium or sodium bicarbonate did not improve the utilization by sheep of high fat rations. As both of these salts are basic and, as shown by these workers, exert a pronounced effect upon ruminal pH, it is certainly feasible to assume that any possible beneficial effects of the cations were completely nullified by the concomitant increase in ruminal pH. Pfander and Phillipson (1953) reported that absorption of volatile fatty acids from the rumen was related to the ruminal pH. By lowering the ruminal pH from 6.4 to 5.0, they found that the absorption of volatile fatty acids was increased from 21 to 98 milliequivalents per hour.

A significant question in ruminant nutrition regards the possible effect of added fat upon the fecal excretion of dietary trace minerals. If fat were added to diets which were on the borderline or slightly deficient in a certain trace cation, it is possible that enough of that particular element would combine with the fatty acids to make the resultant diet greatly deficient and, of course, would result in poorer gains and/or other abnormalities. It is of interest to note that the results of Tefft (1954) bear upon this point. In his studies, the omission of the ferrous ion from a simulated alfalfa ash fed to sheep receiving a high fat diet resulted in weight loss, and the animals became severely anemic, while those animals receiving the whole simulated alfalfa ash were gaining and maintained normal red blood cell count and hemoglobin content.

Summary

Twelve wether lambs each weighing approximately 75 lb. were used to determine the effects of corn oil upon the utilization of dietary calcium and phosphorus. The comparative balance and isotope dilution procedures (calcium-45 and phosphorus-32) were used to determine the fecal endogenous calcium and phosphorus excretions. The inclusion of 7.5% corn oil did not significantly affect the apparent digestibility, fecal endogenous excretion, true digestibility or net retention of dietary phosphorus. Neither did it affect the fecal endogenous or urinary excretion of dietary calcium. This level of fat, however, significantly reduced both the apparent and true digestibilities of dietary calcium, the cumulative result being a decrease in the net retention of dietary calcium which closely paralleled the increase in the fecal excretion of calcium.

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ESTROGENIC ACTIVITY OF SOYBEAN FORAGE[1]

ADEN C. MAGEE AND GENNARD MATRONE
North Carolina State College,[2] Raleigh

REPRODUCTIVE failures have been observed in rabbits fed diets containing large amounts of soybean material (Kendall *et al.*, 1950; Matrone and Smart, 1956), and there is an indication that soybean forage contains a substance(s) which contributes to the failures noted. The specific factor(s) has not been identified, but results of several investigations (Carter *et al.*, 1953; Carter *et al.*, 1955; Matrone *et al.*, 1956; Pope, 1954) suggest the possibility that estrogenic substances of plant origin may be involved. Several legumes have been shown to possess estrogenic activity (Bennetts & Underwood, 1951; Cheng *et al.*, 1953; Curnow *et al.*, 1948; Dohan *et al.*, 1951), and the compounds responsible for the activity of some of the legumes have been isolated (Bradbury and White, 1951; Pope and Wright, 1954). Genistein was found to be the primary estrogenically active compound in subterranean clover, whereas genistin (Cheng *et al.*, 1953a) was found to be the active substance in soybean oil meal. In view of these considerations, the investigation of the estrogenic activity of soybean forage was undertaken.

Experimental Procedure

Green soybean forage, harvested in early bloom stage, was extracted with methanol in a large Soxhlet-type extractor for approximately one week. The residue was discarded, and the methanol solution was treated in a manner similar to the procedure used by Curnow (1954). The methanol solution was extracted with benzene followed by ether. The benzene extract was retained for bioassay, whereas the ether phase was extracted further with saturated aqueous sodium bicarbonate and then with water. The bicarbonate-water washings were combined for bioassay. One aliquot of the washed ether fraction was held for bioassay; another was evaporated to dryness. The resulting residue was dissolved in hot 60% ethanol, treated with charcoal,[3] and filtered. The ethanol filtrate and the charcoal residue were also tested for estrogenic activity.

The investigation consisted of two bioassays. Assay 1, designed to test several fractions described above for estrogenic activity, included benzene, ether, and sodium bicarbonate extracts; the ethanol filtrate; the charcoal residue; three levels of diethylstilbestrol; [4] and a negative control. The

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[2] Animal Nutrition Section, Department of Animal Industry.
[3] Darco decolorizing carbon furnished by Eastman Kodak Company, Rochester, New York.
[4] Supplied by Merck and Company, Rahway, N. J.

crude methanol extract and the methanol fraction (after the benzene and ether extractions) were not included because in preliminary tests mice receiving these materials died before the termination of the assays. Assay 2, designed as a factorial two-dose assay (Bliss, 1952) included two volumes of the ether fraction equivalent to 140 gm. and 280 gm. of soybean forage, respectively; two levels of diethylstilbestrol (0.05 mcg. and 0.1 mcg.); and a negative control.

The fractions were tested for estrogenic activity by the mouse uterine weight technique (Evans *et al.*, 1941). Immature female mice [5] were used

TABLE 1. COMPOSITION OF BASAL DIET USED IN BIOASSAYS

Constituents	Percent
Casein [a]	19
Corn starch [b]	63
Vegetable oil [c]	10
Mineral mix [d]	4
Vitamin mix [e]	2
Cellulose [f]	2

[a] "Vitamin Test", Nutritional Biochemicals Corporation, Cleveland, Ohio.
[b] Corn Products Sales Co., Baltimore, Maryland.
[c] "Crisco", Proctor and Gamble Co., Cincinnati, Ohio.
[d] Salt Mixture W, Nutritional Biochemicals Corporation, Cleveland, Ohio.
[e] 100 gm. vitamin mix contained 1 mg. biotin, 5 mg. folic acid, 0.1 mg. of 0.1% vitamin B_{12} (with mannitol), 25 mg. thiamine, 25 mg. pyridoxine, 50 mg. 2-methyl-naphthoquinone, 50 mg. riboflavin, 50 mg. nicotinic acid, 150 mg. Ca-pantothenate, 5 gm. p-amino benzoic acid, 5 gm. inositol, 7.5 gm. choline, 30 gm. DL-methionine (all contributed by Merck and Company, Rahway, N. J.), and 52 gm. corn starch.
[f] "Alphacel", Nutritional Biochemicals Corporation, Cleveland, Ohio.

in the bioassays, and diethylstilbestrol was used as the reference standard. Individual test rations for each assay were made by mixing each fraction and level of diethylstilbestrol with a basal diet (table 1) according to the method used by Matrone *et al.* (1956). Animals used as controls were fed the basal diet only. The test diets were fed over a period of 5 days in each assay. Twenty-four hours after the last feeding, the animals were sacrificed, and their uteri were removed, pressed lightly on paper towels to remove excess fluid material, and weighed.

Results and Discussion

In assay 1, the mean uterine weight of the mice receiving the ether extract was approximately four times greater than that of the control mice (table 2). A statistical analysis of the data revealed that this difference was highly significant ($P<0.01$). An average two-fold increase in uterine weight in mice given the ethanol fraction was significantly different ($P<0.05$). These treatment differences were still significant after an ad-

[5] Furnished by M. P. Bailey of the North Carolina State Laboratory of Hygiene, Raleigh, N. C.

TABLE 2. ESTROGENIC ACTIVITY OF TREATMENTS TESTED IN ASSAY 1

Treatment	Total dose levels [a]	Mean uterine weight (mg.) [b]
Control	None	6.1
Benzene fraction	280 gm. [c]	5.4
Ether fraction	280 gm. [c]	27.0 [d]
Sodium bicarbonate fraction	280 gm. [c]	6.4
Ethanol filtrate	280 gm. [c]	11.9
Charcoal residue	280 gm. [c]	6.9
Diethylstilbestrol	0.025 mcg.	8.4
	0.050 mcg.	13.7
	0.100 mcg.	43.5

[a] All test material was administered orally.
[b] Each value represents the mean of six animals, unless otherwise indicated.
[c] Amount of forage (fresh weight basis) represented in dose of fraction administered.
[d] Mean of five animals; one animal died before termination of the assay.

justment was made for body weight by covariance, indicating that they were not associated with changes in body weight. From these results, it is apparent that the soybean forage tested contained an estrogenically active substance that was soluble in both ether and ethanol. Other fractions tested in assay 1 were not significantly different from the control, indicating that they had no detectable estrogenic activity.

The mean uterine weights of the mice obtained from the factorial two-dose assay (assay 2) are presented in table 3. Calculation of a potency estimate indicated that 1.0 gm. of green soybean forage (fresh weight basis) was equivalent to approximately 3.38×10^{-4} mcg. of diethylstilbestrol.

Carter *et al.* (1953) estimated the estrogenic potency of genistein as 4.44×10^{-6} times the potency of diethylstilbestrol. On the basis of this

TABLE 3. ESTROGENIC ACTIVITY OF THE ETHER FRACTION OF GREEN SOYBEAN FORAGE AS COMPARED TO DIETHYLSTILBESTROL (ASSAY 2)

Treatment	Total dose levels [a]	Mean uterine weight (mg.) [b]
Control	None	5.5 [c]
Ether fraction	140 gm. [d]	15.5 [c]
	280 gm. [d]	22.4
Diethylstilbestrol	0.05 mcg.	12.2 [c]
	0.10 mcg.	26.9

[a] All test material was administered orally.
[b] Each value represents the mean of six animals, unless otherwise indicated.
[c] Mean of five animals; one animal died before termination of the assay.
[d] Amount of forage (fresh weight basis) represented in the total dose administered to each animal on the treatment.

estimate, it appears that 1.0 mg. of genistein has an estrogenic activity equivalent to 13 gm. of soybean forage (fresh weight basis). Thus, if genistein were the estrogenic-like factor in the forage, it would contain an equivalent of 7.6 mg. of genistein per 100 gm. of fresh matter or approximately 36 mg. of genistein per 100 gm. of dry matter. Carter *et al.* (1955) found that 200 mg. of genistein, the glucoside of genistein, per 100 gm. of diet was required to affect the number of litters dropped by mice. Curnow (1954) reported that the *Dwalganup* strain of subterranean clover contains 740 mg. of genistein per 100 gm. of dry matter which is sufficient to account for the estrogenic activity of the plant and is probably responsible for the infertility in sheep grazing on subterranean pastures.

Pieterse and Andrews (1956) have estimated the potency in terms of diethystilbestrol per 100 gm. of dry matter, of alfalfa, red clover, ladino clover, and birdsfoot trefoil as approximately 0.1–3.6 mcg., 0.1–2.2 mcg., 0.1 mcg., and 0.1 mcg., respectively. By comparison, the estimated potency of the soybean forage obtained in this study is approximately 0.14 mcg. of diethylstilbestrol per 100 gm. of dry matter. That the estrogenic activity of green soybean forage is comparatively low agrees with the results of Pieterse and Andrews (1956) who reported no detectable activity in soybean forage under their conditions. From these considerations it appears unlikely that the factor in soybean forage contributing to reproductive disorders in rabbits is primarily an estrogenic substance.

Summary

An investigation of green soybean forage was conducted for the purpose of determining if this forage had any estrogenic activity. A crude methanol extract of the forage was fractionated, and the fractions obtained were tested for estrogenic activity by the mouse uterine weight technique. Estrogenic activity was detected in green soybean forage. The estrogenically active substance could be removed from the methanol extract of the plant material with ether; it was not soluble in either benzene or aqueous sodium bicarbonate. From 100 gm. of green soybean forage (fresh weight basis), the estrogenic effect on the uterus of the immature mouse was equivalent to the uterine stimulation produced by approximately 0.03 mcg. of diethylstilbestrol.

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RUMINANT UTILIZATION OF SODIUM META-, ORTHO- AND PYROPHOSPHATES

ALLEN D. TILLMAN [1] AND JOHN R. BRETHOUR [2]

University of Tennessee—Atomic Energy Commission Agricultural Research Laboratory, Oak Ridge [3, 4, 5]

THE availability to chicks of phosphorus supplied by meta- or pyrophosphates has been extensively studied (McConnell *et al.*, 1944; Matterson *et al.*, 1945; Bird, *et al.*, 1945; Gillis *et al.*, 1948; Gillis *et al.*, 1954; Motzok *et al.*, 1956); however, only one research paper was found which dealt with this problem in regard to ruminent nutrition. Ammerman *et al.*, (1957), working with sheep, found that the phosphorus of gamma calcium pyrophosphate was almost totally unavailable while that supplied by vitreous calcium metaphosphate appeared to be about 50% as available as that of monocalcium phosphate. These workers also found that gamma calcium pyrophosphate was effective in increasing the plasma inorganic phosphorus levels of the sheep and suggested that the phosphorus of this compound was absorbed and subsequently excreted into the gastrointestinal tract; however, they were not able to study this. Recent advances in radioisotope procedures (Hansard, 1956) permit the separation of fecal endogenous phosphorus from that portion that was not absorbed from the gastrointestinal tract. It thus seemed desirable to determine, using simultaneously chemical and radiochemical balances, the utilization by sheep of monosodium phosphate, vitreous sodium metaphosphate and sodium acid pyrophosphate.

Experimental Procedure

The basal ration, the composition of which is shown in table 1, was similar to that used by Ammerman *et al.*, (1957). The daily rations consisted of 908 gm. of the basal diet plus 1.23 gm. of phosphorus supplied by monosodium phosphate (ration 1), vitreous sodium metaphosphate (ration 2), and sodium acid pyrophosphate (ration 3). The calcium intakes were equalized among all rations by the addition of calcium carbonate.

Eighteen wether sheep approximately 13 months old and weighing 90 to 100 lb., were randomly divided into three groups each of six animals.

[1] Research participant, Oak Ridge Institute of Nuclear Studies, on leave from Oklahoma State University.

[2] Present address, Kansas Agricultural Experiment Station, Hays.

[3] Published with the approval of the Director, Tennessee Agricultural Experiment Station.

[4] The radioactive materials were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission. This study was completed under Contract No. AT-40-1-Gen-242 between the University of Tennessee and the Atomic Energy Commission.

[5] The technical assistance of W. A. Lyke, B. Richmond and G. Tapp is acknowledged and sincerely appreciated.

Each sheep was fed one-half of his daily ration twice daily during a 14-day preliminary period followed immediately by a 7-day collection period. Feces and urine were quantitively collected once daily during the latter period. The sheep were kept during the entire experiment in metabolism stalls described by Briggs and Gallup (1948) and modified for radioisotope

TABLE 1. COMPOSITION OF BASAL DIET

Ingredient	Percent
Corn starch	22.9
Corn sugar	22.9
Wood pulp	20.0
Cottonseed hulls	20.0
Mineral mixture [a]	4.0
Urea [b]	2.0
Drackett C–1 protein	5.0
Cottonseed oil	3.0
Choline chloride	0.10
Vitamins A and D [c]	0.1
Vitamin E	+
Vitamin mixture [d]	+
Calcium content	0.090
Phosphorus content	0.069

[a] The percentage composition was as follows: $NaSO_4$, 34.0; $K_2C_6H_5O_7 \cdot H_2O$, 33.8; iodized NaCl, 15.4; $CaSO_4 \cdot 2H_2O$, 7.5; $MgCO_3$, 5.83; $FeC_6H_5O_7 \cdot 6H_2O$, 2.36; $MnSO_4 \cdot 6H_2O$, 0.122; $CuSO_4 \cdot 5H_2O$, 0.029; $CoCl_2 \cdot 6H_2O$, 0.24; $ZNCl_2$, 0.020; CaF_2, 0.020; and MoO_3, 0.003.

[b] Feeding-grade urea, containing 42.13% nitrogen, supplied through courtesy of Dr. David Green, Allied Chemical and Dye Corporation, New York, N. Y.

[c] Quadrex, NOPCO Chemical Co., Harrison, New Jersey. It contained 20,000 I.U. of vitamin A and 2500 U.S.P. units of vitamin D_3 per gram, and was supplied through courtesy of Dr. M. Hochberg.

[d] Vitamins added in mg. per lb. of ration: Ascorbic acid, 14.0; biotin, 0.03; calcium pantothenate, 27.0; folic acid, 0.2; inositol, 90.0; niacin, 18.0; p. aminobenzoic acid, 10.0; pyridoxine HCl, 2.25; riboflavin, 4.5; thiamin-HCl, 2.25; 2-methyl-1, 4-naphthoquione, 0.9; and vitamin B_{12}, 0.015.

studies by Hansard *et al.* (1951). At the beginning of the collection period, three animals from each group were given one millicurie of phosphorus-32 by intravenous injection, while the remaining sheep in each group were given the same dosage orally. Calculations of fecal endogenous phosphorus were made by the isotope dilution method of Kleiber *et al.* (1951), which was modified to allow a single injection of the isotope (Hansard, 1956), and by the comparative balance technique as described by Hansard (1952) and Comar *et al.* (1953). Blood samples were taken from all animals by jugular puncture at frequent intervals during the collection period for the determination of calcium and phosphorus and radiophosphorus. Calcium was determined by the method of Clark and Collip (1925). Inorganic phosphorus analyses were made essentially by the method of Fiske and Subbarow (1925). Radioassay of phosphorus-32 was performed by using a thin window Geiger-Mueller counter connected to a scaler. Statistical treatment of the data was by the analysis of variance method.

Results and Discussion

The results of this trial are shown in table 2. The fecal calcium excretion did not differ significantly among the phosphorus sources. As differences between urinary calcium excretions due to treatment were

TABLE 2. DAILY BALANCES OF CALCIUM AND PHOSPHORUS

Item	Ration 1 Monosodium phosphate	Ration 2 Vitreous sodium metaphosphate	Ration 3 Acid sodium pyrophosphate
Calcium			
Intake, gm.	2.790	2.790	2.790
Basal diet, gm.	0.820	0.820	0.820
Calcium carbonate, gm.	1.970	1.970	1.970
Excretion, gm.	2.017	2.242	2.063
Feces	1.983	2.204	2.008
Urine	0.034	0.038	0.055
Retained, % of intake	27.7	19.6	26.1
Apparent digestibility, %	28.9	21.0	28.0
Phosphorus			
Intake, gm.	1.860	1.860	1.860
Basal diet, gm.	0.630	0.630	0.630
Supplement, gm.	1.230	1.230	1.230
Excretion	1.259	1.542*	1.215
Feces	1.181	1.453*	1.155
Urine	0.078	0.095	0.060
Fecal erdogenous, gm.	0.968	1.219*	0.978
Retained, % of intake	32.3	16.8	34.7
Apparent digestibility, %	36.5	21.9*	37.9
Endogenous, % of intake	52.0	65.5*	52.6
Endogenous, % of fecal	82.0	83.9	84.7
True digestibility,%	88.6	87.4	90.4

* Indicates treatment differences significant at the 5% level.

small, the retentions of dietary calcium in all treatments closely paralleled the apparent digestibilities of this element.

When phosphorus was considered, it was found that the differences between monosodium phosphate and acid sodium pyrophosphate were small, indicating that this form of pyrophosphate was as available to sheep as was the orthophosphate. This observation agrees with those of Gillis *et al.* (1948) (1954), and Motzok *et al.* (1956), who used chicks as experimental animals. As each supplement in the present experiment supplied 66.1% of the dietary phosphorus in rations supplying a daily allowance of only 2.0 gm. per 100 lb. of body weight, it appears that both of these compounds furnished dietary phosphorus which was available for utilization by the sheep.

Fecal phosphorus excretion appeared to be greater ($P<0.05$) when vitreous sodium metaphosphate was fed in place of monosodium phosphate. Ammerman *et al.* (1957), using calcium metaphosphate, also found this to be true. Urinary phosphorus excretions in all treatments were small; thus, the net retentions of phosphorus closely paralleled in all treatments the apparent absorption of this element. In this connection Ammerman *et al.* (1957), working with cattle and sheep, also noted that urinary phosphorus values were small and suggested that these values could be ignored in most studies of this nature. Even though the results of the present experiment tend to support this idea, it must be emphasized that variation between animals receiving the same treatment is large and that more critical results could be obtained if the urine were collected and analyzed for its phosphorus content.

Fecal endogenous (metabolic) phosphorus excretion was significantly ($P<0.05$) increased when vitrous sodium metaphosphate was fed. The results in table 2 show that endogenous phosphorus accounted for the increased fecal phosphorus noted for this group, suggesting that the phosphorus of sodium metaphosphate was absorbed from the gastrointestinal tract but was subsequently excreted and appeared in the feces. Ammerman *et al.* (1957) also reported results that support this idea. They found in balance studies that both vitreous calcium metaphosphate and gamma calcium pyrophosphate were poor sources of phosphorus, but that both of these compounds caused significant increases in plasma inorganic values of sheep previously fed a very low phosphorus basal diet. The results of Long *et al.* (1956) also bear on this point. They noted that soft phosphorus with colloidal clay was ineffective in promoting growth of animals previously fed a very low phosphorus basal diet but that it was effective in increasing the plasma inorganic phosphorus level. In the present experiment, frequent blood samples were taken from the animals during the 7-day collection period; however, differences in plasma phosphorus due to treatment were small and within the range of experimental error. These animals, however, had not been on a phosphorus deficient diet prior to the initiation of the experiment.

The reason(s) for the poor utilization of vitreous sodium metaphosphate in the present study and the phosphates used by Ammerman *et al.*, (1957) has not been revealed. They reported that gamma calcium pyrophosphate and vitreous calcium metaphosphate had low availability, and suggested that the difficulty lies in the problem of solubility and absorption, or in the inability of the animal to utilize efficiently that which is absorbed. Our results obtained with vitreous sodium metaphosphate lend support to the latter idea.

Summary

Eighteen wether sheep approximately 13 months old and weighing 90–100 lb. were used to determine the availability of phosphorus sup-

plied as vitreous sodium metaphosphate and acid sodium pyrophosphate; monosodium phosphate was used as the control. All phosphorus supplements supplied 66.1% of the phosphorus in rations supplying 2.0 gm. of phosphorus per 100 lb. of liveweight. Criteria of response were apparent digestibilities, net retentions, fecal endogenous excretions and true digestibilities of phosphorus. Acid sodium pyrophosphate was equally as available as monosodium phosphate. Vitreous sodium metaphosphate caused increased fecal phosphorus excretion; but, as this could be accounted for by increased endogenous phosphorus, the true digestibilities of the three phosphates were not different. The data indicate that although the phosphorus of vitreous sodium metaphosphate was absorbed, it was inefficiently utilized.

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EFFECT OF SUPPLEMENTAL FAT ON DIGESTION AND THE RUMINAL CALCIUM REQUIREMENT OF SHEEP [1, 2]

T. W. WHITE,[3] R. B. GRAINGER, F. H. BAKER AND J. W. STROUD

Kentucky Agricultural Experiment Station, Lexington

VAST quantities of potential energy are available for utilization by the ruminant in the cellulose of low-quality roughage and in surplus low-cost fats. Brooks *et al.* (1954), Rhodes *et al.* (1956), Ward *et al.* (1957), and Summers *et al.* (1957) reported a reduction in digestion of ration nutrients by sheep fed low-quality roughage rations containing 3% supplemental fat. An increase in the fat content of sheep rations from 2.8 to 6.4% by addition of corn oil increased the digestibility of feed constituents (Swift *et al.*, 1947); however, a further increase in fat content to 9.8% reduced digestibility of all constituents except ether extract. It should be noted that the rations used by Swift and co-workers contained mixed hay (timothy and alfalfa) as roughage.

Brooks *et al.* (1954), Rhodes *et al.* (1956), Ward *et al.* (1957) and Summers *et al.* (1957) reported that the presence of alfalfa ash in sheep rations alleviated the depressing effect of supplemental fat on cellulose and protein digestibility. Grainger *et al.* (1957) observed that a trace mineral mixture containing Fe, Cu, Co, Mn, Zn and Mo did not alleviate the depressing effect of supplemental fat on organic matter or cellulose digestibility. Ellis, *et al.* (1958) reported an improvement in cellulose digestion due to the presence of molybdenum in a semi-purified ration which contained 2% lard.

The experiments reported herein were designed, (1) to investigate the effect of prolonged fat supplementation on digestion, and possible recovery prior to or following a ration change, (2) to ascertain the active component(s) in alfalfa ash that reverse the depressed digestibility observed with rations which contain supplemental fat, and (3) to study fat-mineral interrelationships.

Experimental

Crossbred wethers which weighed about 80 lb. each were treated for internal parasites with phenothiazine and sheared prior to this series of

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[3] Ralston-Purina Fellow. Present Address: Animal Husbandry Department, University of Missouri, Columbia, Missouri.

trials. In all trials, four wethers were randomly assigned to each treatment. During the periods between trials (30 days), the wethers were fed alfalfa hay *ad libitum*. The semi-purified basal rations and their calculated chemical composition are presented in table 1. These calculations were based on the chemical composition of the ration ingredients as determined in this laboratory.

TABLE 1. AVERAGE DAILY RATIONS AND THEIR CHEMICAL COMPOSITIONS

Items	Ration A	Ration B
Ration constituent, gm.		
Ground corncobs	455.0	——
Cottonseed hulls [a]	——	546.0
Urea [a]	8.4	8.4
Drackett protein	42.3	42.3
Corn syrup	70.0	27.0
Cornstarch	109.4	67.3
Dicalcium phosphate	9.6	9.6
Iodized salt	3.5	3.5
Magnesium sulfate	1.5	1.5
Sulfur (elemental)	0.1	0.1
Vitamin A and D concentrate [b]	0.2	0.2
Dry matter, %	92.4	90.9
Composition of dry matter, %		
Organic matter	96.5	95.2
Protein	11.0	12.1
Ether extract	0.2	1.3
Ash	3.5	4.8
Crude cellulose	30.4	46.4

[a] The cottonseed hulls and urea were supplied by the Buckeye Cellulose Corporation, Cincinnati, Ohio, and Allied Chemical and Dye Corp., New York, respectively.
[b] 10,000 I.U. of vitamin A and 500 I.U. of vitamin D per gm.

The experimental rations, trace mineral mixture and alfalfa ash were prepared as described by Summers *et al.* (1957). The metabolism cages were similar to those described by Briggs and Gallup (1949). Experimental procedures were similar to those described by Woods *et al.* (1956). Nitrogen, dry matter and protein were determined by Official Methods (1950). Crude cellulose, referred to as cellulose, was determined by the method of Crampton and Maynard (1938). The statistical analysis (analysis of variance) was conducted as described by Snedecor (1956) and treatment comparisons by the multiple range test outlined by Duncan (1955).

Trial 1 consisted of two experimental phases. In phase 1, the wethers of group I received ration A and those in group II received ration A in

which 5% corn oil replaced 5% corn syrup. A ration adjustment period was followed by a 10-day preliminary period and three consecutive 10-day collection periods. In phase 2, the wethers of groups I and II were fed ration B in which the crude fiber was known to be less digestible than in ration A (Grainger *et al.*, 1956). At the termination of phase 1, a 3-day ration adjustment period was followed by three consecutive 7-day collection periods. In trials 2 and 3, the ration adjustment period was followed by a 10-day preliminary and a 10-day collection period.

Results and Discussion

Brooks *et al.* (1954), Summers *et al.* (1956) and Ward *et al.* (1957) by the use of the conventional 10-day balance trial have demonstrated the depression of crude fiber or cellulose digestibility by supplemental fat. In trial 1 (table 2) 5% corn oil significantly depressed cellulose diges-

TABLE 2. MEAN APPARENT DIGESTION COEFFICIENTS AND NITROGEN BALANCE (TRIAL 1)

Group number	Treatment	Cellulose [a]	Protein	Daily nitrogen balance
				gm.
		Period I (10 days) [b]		
I	Ration A	71.4	69.5	1.5
II	Ration A + 5% corn oil	58.5	67.0	0.6
		Period II (10 days)		
I	Ration A	70.7	67.7	2.5
II	Ration A + 5% corn oil	45.1	67.3	1.5
		Period III (10 days)		
I	Ration A	67.7	67.5	1.6
II	Ration A + 5% corn oil	39.7	66.1	0.8
		Period IV (7 days) [c]		
I	Ration B	61.2	58.9	2.5
II	Ration B	39.3	55.1	2.0
		Period V (7 days)		
I	Ration B	55.5	53.8	2.6
II	Ration B	49.4	51.9	3.6
		Period VI (7 days)		
I	Ration B	55.4	54.6	2.8
II	Ration B	54.6	54.1	3.1

[a] Cellulose digestion coefficients for Group I were significantly higher than those for Group II in Periods, I, II, III and IV ($P<0.01$).

[b] The cellulose digestion coefficient for Group II in Period I was significantly higher than for Group II in Periods II and III ($P<0.01$).

[c] The cellulose digestion coefficient for Group II in Period IV was significantly lower than for Group II in Period V ($P<0.05$) and in Period VI ($P<0.01$). The cellulose digestion coefficient for Group I in Period IV was significantly higher than for Group I in Periods V and VI ($P<0.01$).

tion in the first three experimental periods, the depression being progressive over the total 30-day collection period. Less than 50% of the available cellulose in ration A was digested by the wethers in group II during period III. At the end of period III the wethers had been on their respective rations for 40 days, and at that time both experimental groups were shifted to ration B (phase 2). In period IV the wethers of group I made the expected adjustment in the level of cellulose digestion whereas those in group II gave no evidence of such an adjustment. Cellulose digestion by group II was significantly increased in periods V and VI over period IV; it was significantly decreased by group I after the shift in roughage source from corncobs to cottonseed hulls. Ten days after the ration change of group I (period V), cellulose digestibility was stabilized at the level shown in period VI. Complete recovery was not obtained in group II until 17 days after the ration change. The mean cellulose digestibility of both groups approached 55% about 24 days (period VI) after the ration change.

Brooks *et al.* (1954) Ward *et al.* (1957) and Pfander *et al.* (1957) postulated that the effect of supplemental fat on crude fiber digestibility was produced by coating the fibrous portion of the ration. This hypothesis is not supported by the findings of Brethour, *et al.* (1958) wherein mixing animal fat in only the concentrate portion of a ration did not reduce its unfavorable influence on ruminant performance. In periods I, II and III the ratio of fat to cellulose in the ration was constant; however, there was a progressive depression of cellulose digestion rather than an initial depression with no further decrease. A purely physical effect on cellulose digestion would be alleviated by passage of the fat-coated cellulose from the rumen. However, recovery from the decreased cellulose digestibility did not occur until after 17 days. These data suggest that supplemental fat decreases certain microbial metabolic activity and/or modifies the rumen microbial population concerned with cellulose digestion.

Brooks *et al.* (1954) and Summers *et al.* (1957) reported reversal of the corn oil depression of crude fiber or cellulose digestion by addition of alfalfa ash to experimental rations containing cottonseed hulls or ground corncobs as sources of roughage. In trial 2 (table 3), the addition of 4.1% alfalfa ash to a ration that contained 5% corn oil completely reversed the depression of cellulose digestibility. Phosphoric acid (0.86 gm. P) or a trace mineral mixture formulated to contain an amount of Fe, Cu, Mn, Co, Zn, Mo and B equivalent to that in 30 gm. of alfalfa ash (Shrenk and Silker, 1951) had no effect on digestibility of cellulose in a ration that contained 5% corn oil. However, 4.4 gm. Ca ($CaCO_3$) or 4.4 gm. Ca and 0.86 gm. P counteracted the depressing effect of the corn oil. Trial 3 (table 4) verified the findings in trial 2 and indicated that the basal ration was adequate in Ca and P for optimum cellulose utilization. As observed by Brooks *et al.* (1954) corn oil depressed pro-

TABLE 3. MEAN APPARENT DIGESTION COEFFICIENTS AND NITROGEN BALANCE (TRIAL 2)

Treatment	Cellulose [a]	Protein [b]	Daily nitrogen balance
			gm.
Ration A (RA)	77.8	79.0	2.1
RA+5% corn oil (CO)	67.7**	76.8	1.6
RA+5% CO+30 gm. alfalfa ash	76.3	77.8	2.6
RA+5% CO+trace minerals [c]	65.8**	77.6	1.7
RA+5% CO+0.86 gm. P	69.0**	77.2	1.6
RA+5% CO+4.4 gm. Ca	76.0	79.5#	2.8
RA+5% CO+0.86 gm. P+4.4 gm. Ca	77.5	80.2#	2.2

[a] ** designates a cellulose digestion coefficient which is significantly lower than those coefficients that are not so marked ($P<0.01$).

[b] # designates a protein digestion coefficient which is significantly higher than the coefficient for the ration supplemented only with corn oil ($P<0.05$).

[c] Contained Fe, Cu, Mn, Co, Zn, Mo and B equivalent to amount contained in 30 gm. alfalfa ash.

tein digestibility and this depression was reversed by addition of Ca or Ca and P.

Under the experimental conditions of trials 2 and 3, the complete effect of alfalfa ash on cellulose digestion was produced by the amounts of Ca, or Ca and P present in alfalfa ash. These data indicate that for optimum cellulose utilization the ruminal requirement for Ca was increased in the presence of supplemental fat. The reason for this was not apparent from the data. However, Pfander *et al.* (1957) reported hydrogenation of corn oil by ruminants 5 hours after feeding and Swell *et al.* (1956) reported that corn oil or olive oil stimulated formation of a calcium-lipid-

TABLE 4. MEAN APPARENT DIGESTION COEFFICIENTS AND NITROGEN BALANCE (TRIAL 3)

Treatment	Cellulose [a]	Protein [b]	Daily nitrogen balance
			gm.
Ration A (RA)	75.8	74.3#	3.6
RA+5% corn oil (CO)	62.3**	70.9	3.2
RA+5% CO+4.4 gm. Ca	71.0	72.7	3.9
RA+5% CO+4.4 gm. Ca+0.86 gm. P	72.8	75.3##	4.3
RA+4.4 gm. Ca	75.7	73.1	3.5
RA+4.4 gm. Ca +0.86 gm. P	76.1	72.9	5.4

[a] ** designates a cellulose digestion coefficient that is significantly lower than all other cellulose digestion coefficients ($P<0.01$).

[b] # and ## designate protein digestion coefficients which are significantly higher than the coefficient for the ration supplemented only with corn oil ($P<0.05$) and ($P<0.01$) respectively.

phosphate complex in rats. Calverley *et al.* (1949) reported that soap excretion was increased when hydrogenated cottonseed oil was fed to rats. These findings may possibly explain the Ca-fat relationship shown in trials 2 and 3.

Summary

Three balance trials were conducted with mature wethers to determine, (1) the effect on cellulose digestibility of prolonged supplementation of a ration with fat, and the subsequent rate of recovery prior to or following a ration change, and (2) the active component(s) in alfalfa ash that reverse the depressed digestibility observed with rations which contain supplemental fat. Five percent corn oil progressively decreased cellulose digestion during the first 40 days of trail 1. Recovery of cellulose digestion was not complete until 17 days after a ration change and the omission of corn oil. In trials 2 and 3 the effect of 30 gm. of alfalfa ash in restoring digestion of cellulose in a ration that contained 5% corn oil was produced by 4.4 gm. Ca or 4.4 gm. Ca and 0.86 gm. P. Phosphorus (0.86 gm.) alone or a trace mineral mixture which contained Cu, Mo, Mn, Co, Fe, Zn and B was ineffective in this regard.

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UTILIZATION OF TOCOPHEROL FROM ARTIFICIALLY DEHYDRATED ALFALFA BY THE HOLSTEIN CALF[1]

H. D. Eaton, R. Teichman, J. E. Rousseau, Jr.,[2] Martha W. Dicks, A. P. Grifo, Jr., C. F. Helmboldt, E. L. Jungherr, and L. A. Moore

Storrs (Conn.) Agricultural Experiment Station[3] *and U. S. Department of Agriculture*[4]

ALTHOUGH artificially dehydrated alfalfa has long been recognized as an excellent source of carotene, provitamin A, for livestock, until recently little attention has been focused on the content and availability to livestock of the tocopherol in this material. The studies of Brown (1953), Kohler *et al.* (1955), and Ames (1956) have indicated that artificially dehydrated alfalfa contains a relatively high concentration of tocopherol in comparison to most other feedstuffs and that the tocopherol exists in the α-form which exhibits greater biological activity, on the basis of rat and chick data, than the other forms of tocopherol. While no quantitative data with respect to the availability of tocopherol from artificially dehydrated alfalfa to livestock were found in the literature, experiments with chicks (Singsen *et al.*, 1955; Bunnell, 1957) have demonstrated in general, lower utilization of the tocopherol from alfalfa than from d-α-tocopherol or d-α-tocopheryl acetate *per se*. The present study was undertaken to determine quantitatively the utilization of tocopherol from artificially dehydrated alfalfa by Holstein calves, employing blood plasma and tissue concentrations of tocopherol as criteria.

Experimental Procedure

Animals and Rations. Forty 1-day old Holstein male calves from various Connecticut State Institution herds, during the period October 1955 to January 1956, were brought to the research barn and placed in individual tie stalls. Upon arrival, each calf received one 500-milligram oblet of

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[2] Present address: U. S. Fishery Products Lab., Ketchikan, Alaska.

[3] Animal Industries and Animal Diseases Departments (C.F.H. and E.L.J.).

[4] Div. of Nutrition and Physiology, Dairy Cattle Research Branch, Agricultural Research Service (L.A.M.).

chlortetracycline and 200,000 U.S.P. units of Vitamin A from a feeding oil, containing 25% lecithin by weight. Thereafter, each calf was raised to 63 days of age on a standard regimen of limited whole milk, limited starter and *ad libitum* U. S. no. 2 mixed hay. On the 64th day of age each calf was fed an intake of a basal vitamin A depletion ration (a mixture by weight of one-third of dried beet pulp and two-thirds of a grain mixture composed of the following: 419.5 lb. ground barley, 500 lb. crimped oats, 500 lb. wheat bran, 150 lb. linseed oil meal—expeller process, 150 lb. soybean oil meal—solvent process, 200 lb. cane molasses, 40 lb. 500-potency B-Y dried fermentation solubles, 20 lb. steamed bone meal, 20 lb. iodized salt, and 0.5 lb. irradiated yeast—Standard Brands type 9-F, per ton of mixture) which was relatively low in tocopherol content, to provide an anticipated live weight increase of 10 lb. per 7-day period. Vitamin A from a dry carrier (NOPCAY 250 Type M, Lot V6659, 250,000 U.S.P. units per gram) was fed at the daily rate of 40 mcg. per lb. of live weight. The vitamin A concentrate was adjusted to approximately 10,000 U.S.P. units per gram by premixing with finely ground basal ration.

On the 92nd day of age, each calf was fed in addition to the basal ration, one of five levels of tocopherol from a dry carrier (American Cyanamid Co. vitamin E feed supplement, 20,000 I.U. vitamin E per lb. A concentrate of d-α-tocopheryl acetate absorbed on solvent extracted soybean flakes), to provide 0.000, 0.128, 0.256, 0.512 or 1.024 mg. of free tocopherol per lb. live weight per day or one of five levels of tocopherol from artificially dehydrated alfalfa leaf meal (obtained from the Nat. Alfalfa Dehydrating and Milling Co., Kansas City, Mo. and containing no additives such as antioxidents, oils, etc.), 0.000, 0.125, 0.250, 0.500, and 1.000 mg. per lb. live weight per day, for a 12-wk. period. Also the vitamin A intake was increased so that each calf fed the tocopherol from the dry carrier received a daily intake of vitamin A from a dry carrier of 60 mcg. per lb. of live weight and each calf fed tocopherol from the alfalfa received the same daily intake of vitamin A, but the amount of vitamin A from the dry carrier was reduced in proportion to the amount of the pro-vitamin, carotene, contained in the artificially dehydrated alfalfa leaf meal (based on previously established carotene-vitamin A equivalencies for the Holstein calf, Rousseau *et al.*, 1956). The first 10 calves to arrive at the research barn were assigned randomly to one of the 10 treatments as outlined above and this procedure was repeated for a total of 40 calves. Each calf was slaughtered upon completion of the 12-wk. tocopherol supplementation period which was from the 91st to the 176th day of age.

Intake of the basal ration, calculation and incorporation of the vitamin supplements or alfalfa into the basal ration, treatment for scours, and other management procedures were identical to those previously reported, Rousseau *et al.* (1954). Average minimum and maximum daily temperatures for the tocopherol supplementation period with their standard errors

were, respectively, 50.2±0.4° F and 61.4±0.5° F. Average light intensity measured at a height of 48 in. in the center of each calf stall, at successive 28-day periods, was 4.8±0.4 foot candles.

Observations and Analyses. All feeds fed and refused, except the vitamin supplements were weighed to the nearest 0.1 lb. The artificially dehydrated alfalfa leaf meal was weighed to the nearest 0.1 gram and the dry carriers of tocopherol and vitamin A to the nearest 0.01 gram. Live weights were determined at successive 7-day age periods throughout the experiment and linear growth measurements, height at withers, heart girth and circumference of barrel at the beginning and termination of the 12-wk. tocopherol supplement period.

TABLE 1. AVERAGE CHEMICAL COMPOSITION OF FEEDS FED

	Per cent dry matter[a]	Per cent of dry matter[a]					
		Crude protein	Ether extract	Crude fiber	N.F.E.	Ash	Carotene mg./lb.
Mixed hay, chopped	93.08	9.82	1.72	32.29	49.08	7.09	8.46
	0.25	0.30	0.14	0.34	0.16	0.14	1.46
Calf starter	92.56	20.86	3.32	5.11	62.96	7.76	0.17
	0.58	0.07	0.30	1.09	0.99	0.09	0.02
Vitamin A depletion mixture	90.73	16.36	2.57	13.03	60.96	7.07	0.09
	0.68	0.39	0.16	0.30	0.52	0.16	0.03
Dry carrier of tocopherol	90.92	52.14	10.35	2.60	28.87	6.04	....
	0.69	0.37	0.89	0.08	0.67	0.07	....
Dehydrated alfalfa leaf meal	92.60	23.66	4.58	18.89	40.76	12.10	113.77
	0.71	0.51	0.23	0.53	0.46	0.24	3.74

[a] Standard error of the mean is given below each value.

Venous blood samples were obtained by jugular puncture the day prior to supplementation with tocopherol and at biweekly periods thereafter during the 12-wk. tocopherol supplementation period. Plasma tocopherols were determined as previously described (Rousseau *et al.*, 1957).

Samples of the liver, heart (ventricular muscle), trapezius muscle and perinephric fat were obtained at slaughter and held at 0° F until analyzed for lipids and tocopherol as previously described for the liver (Rousseau *et al.*, 1957*a*), except that the perinephric fat samples were saponified (Eggitt and Ward, 1953) prior to molecular distillation and chromatography. Dry matter was determined by drying at 100° C for 24 hours. For histologic study, sections of the heart and trapezius muscle were obtained at slaughter and prepared as previously described (Rousseau *et al.*, 1957).

Samples of all feeds fed, artificially dehydrated alfalfa leaf meal and vitamin supplements were obtained at 28-day periods and analyzed for proximate constituents by A.O.A.C. (1955) procedures, table 1. The artificially

dehydrated alfalfa leaf meal contained 250.6±8.2 micrograms of carotene per gram, according to methods previously described (Rousseau *et al.*, 1954) and 0.195±0.005 milligrams of tocopherol (as d-α-tocopherol) per gram (Rousseau *et al.*, 1958); the dry carrier of d-α-tocopheryl acetate contained 33.2±0.7 milligrams of free tocopherol per gram, (Rousseau *et al.*, 1957); and the dry carrier of vitamin A 69,779±1,367 micrograms of vitamin A (as alcohol) per gram (Rousseau *et al.*, 1957).

Statical procedures as outlined by Cochran and Cox (1957), Finey (1952), and Snedecor (1956) were used in the analyses of the data.

Results and Discussion

Feed Consumption, Health and Growth. Calves readily consumed their basal ration allowance plus vitamin supplements. The number of days each calf refused to completely consume its allowance during the 12-wk. tocopherol supplement period averaged less than one day. Similarly, the incidence of diarrhea was extremely low, averaging 0.4 days per calf.

The average initial growth measurements, determined the day prior to tocopherol supplementation, with their standard deviations per calf were 212±13 lb. for live weight, 35.0±0.9 in. for height at withers, 38.9±1.0 in. for heart girth, and 48.1±2.0 in. for circumference of barrel. Average increases during the 12-wk. tocopherol supplement period of these growth criteria were, respectively, 166±11 lb., 5.7±0.9 in., 8.6 ±1.2 in., and 10.5±1.8 in.

Tissue Tocopherol Concentrations. Table 2 contains the initial plasma tocopherol concentrations determined the day prior to the addition of tocopherol to the basal ration from the dry carrier or from the artificially dehydrated alfalfa, the average plasma tocopherol concentrations during the 12-wk. tocopherol supplementation period and the tocopherol concentrations of the liver, heart, trapezius muscle, and perinephric fat, expressed on a wet basis and on a per gram of lipid basis, at the termination of the tocopherol supplement period. In general, all tissues exhibited an increase in total tocopherol concentration with an increase in tocopherol intake. With the exception of the trapezius muscle, this rate of increase was considerably less in the case of plasma than for the other tissues.

To evaluate quantitatively the relative availability of tocopherol from the dry carrier and from the artificially dehydrated alfalfa, slope-ratio bioassay procedures (Finney, 1952) were used. In plotting the individual tissue tocopherol concentrations against tocopherol intake for each source of the vitamin, it was observed that the variation in the tissue concentrations of tocopherol increased with an increase in tocopherol intake. Standard deviations were found to be approximately proportional to the mean concentration from which each was derived (i.e., coefficients of variation were essentially of the same magnitude and exhibited no consistent trend with an increase in tocopherol concentration), thus indicating

TABLE 2. EFFECT OF TOCOPHEROL INTAKE FROM DRY CARRIER AND FROM ARTIFICIALLY DEHYDRATED ALFALFA UPON TISSUE CONCENTRATIONS OF TOCOPHEROL[a]

Tocopherol added to basal ration	Plasma		Liver		Heart		Trapezius muscle		Perinephric fat	
	Initial value	Av. during supplementation	Wet basis	Lipid basis	Wet basis	Lipid basis	Wet basis	Lipid basis	Wet basis	Lipid basis
mg./lb. live weight/d.	mg./100 ml.		mg./100 g.	mg./g. lipid	mg./100 g.	mg./g. lipid	mg./100 g.	mg./g. lipid	mg./100 g.	mg./g. lipid
Dry carrier										
0.000	0.05	0.06	0.08	0.02	0.12	0.04	0.14	0.12	1.02	0.01
0.128	0.06	0.10	0.20	0.05	0.24	0.08	0.20	0.16	1.32	0.02
0.256	0.06	0.10	0.19	0.05	0.32	0.11	0.21	0.15	1.24	0.02
0.512	0.08	0.16	0.31	0.08	0.60	0.21	0.26	0.22	2.10	0.02
1.024	0.05	0.17	0.54	0.14	0.78	0.28	0.37	0.32	2.37	0.03
Artificially dehydrated alfalfa										
0.000	0.06	0.05	0.08	0.02	0.16	0.05	0.18	0.14	0.98	0.01
0.125	0.07	0.09	0.17	0.04	0.31	0.11	0.24	0.18	1.28	0.01
0.250	0.08	0.12	0.23	0.06	0.41	0.14	0.29	0.23	1.40	0.02
0.500	0.08	0.13	0.26	0.07	0.47	0.16	0.28	0.24	1.52	0.02
1.000	0.05	0.20	0.36	0.10	0.66	0.23	0.32	0.28	1.92	0.02

[a] The results for each level and source of tocopherol represent an average value obtained from four calves.

the need for logarithmic transformation for the purposes of minimizing differences in the variation or a weighting procedure to account for the differences in variation so that analysis of variance procedures, application of tests of significance and derivation of confidence limits would be valid. The use of the logarithmic transformation was deemed inadvisable because Ames and Harris (1956) have found that when the negative control values are not or do not approach zero magnitude, curvature is introduced in the resultant log response—log intake parallel line assay. This was confirmed with the present data. Therefore, a weighting procedure was followed which consisted of weighting each value by the reciprocal of the square of its mean concentration (standard deviation approximately proportional to the mean from which it was calculated; therefore, the square of the mean is approximately proportional to its variance). From the first weighting, new mean concentration values were derived from the regression of concentration on intake, and these were used in the next iteration. This procedure was followed until little or no change occurred in the magnitude of the estimated regression coefficients.

Table 3 contains the regressions of tissue tocopherol concentration on tocopherol intake, the percent variability accounted for by the regressions and the percentage availability of the tocopherol from alfalfa as compared to that from the dry carrier with 95% confidence limits for these percentages.

These regressions, with the exception of the trapezius muscle, appeared to fit the data reasonably well as indicated by the percentages of variation accounted for by the regressions. The wide variation of the different tissues in the percentage availability values for tocopherol from artificially dehydrated alfalfa, 65 to 116% on a wet basis with an unweighted average of 92% and 65 to 115% on a lipid basis with an unweighted average of 89%, was cause for some concern in the interpretation of the results. However, considering these percentage availability values *in toto*, and giving less weight to the trapezius muscle data (because of the poor fit of their regressions in contrast to the others), it would appear reasonable tentatively to assume that the total tocopherol in artificially dehydrated alfalfa as determined chemically, is not as available to the dairy calf as the tocopherol from a dry carrier. Two possible explanations are evident for this difference in availability. One, that the tocopherol in alfalfa is in the alcohol or free form, while in the dry carrier it exists as the ester, acetate. Therefore, one would anticipate greater destruction in the alimentary tract of the alcohol form once it is freed from the alfalfa than in the case of the ester as has been indicated in work with rats by Harris and Ludwig (1949). Two, that the alfalfa is incompletely digested by the dairy calf and thus all the chemically determined tocopherol of alfalfa is not available to the calf.

These tentative findings that the total tocopherol determined chemically in alfalfa is not as available to the calf as tocopherol in a dry car-

TABLE 3. REGRESSIONS OF TISSUE TOCOPHEROL CONCENTRATION ON TOCOPHEROL INTAKE AND RELATIVE AVAILABILITY OF TOCOPHEROL FROM ARTIFICIALLY DEHYDRATED ALFALFA AS COMPARED TO A DRY CARRIER

Tissue	Regression of tissue concentration on intake [a]				
	Common intercept	Rate of change for dry carrier	Rate of change for art. dehydrated alfalfa	Percent of variation accounted for by regression [b]	Percentage availability of tocopherol from art. dehydrated alfalfa [c]
	A	B_1	B_2	%	%
Plasma	0.05	0.543	0.553	90	102 78 to 132 [d]
Liver					
Wet basis	0.10	0.449	0.347	86	77 76 to 79
Lipid basis	0.02	0.122	0.098	86	80 74 to 85
Heart					
Wet basis	0.16	0.687	0.678	90	99 98 to 100
Lipid basis	0.05	0.246	0.237	89	96 93 to 100
Trapezius muscle					
Wet basis	0.18	0.168	0.196	72	116 112 to 121
Lipid basis	0.14	0.157	0.180	82	115 110 to 119
Perinephric fat					
Wet basis	1.08	1.448	0.938	88	65 64 to 65
Lipid basis	0.01	0.017	0.011	89	65 37 to 102

[a] Weighted regressions of the general form $\hat{Y}=A+B_1X_1+B_2X_2$ in which $\hat{Y}$=tissue concentration, A=common intercept or tissue concentration when intake of tocopherol from both sources was zero, B_1=rate of change of tissue concentration for each unit intake of tocopherol from the dry carrier, X_1, and B_2=rate of change of tissue concentrations for each unit intake of tocopherol from the artificially dehydrated alfalfa, X_2. $\hat{Y}$, in the case of plasma, was mg. tocopherol/100 ml. and in the case of the remaining tissues, $\hat{Y}$ was mg. tocopherol/100 g. for the wet basis and mg. tocopherol/g. lipid for the lipid basis. X_1 and X_2, in the case of plasma, were log [(mg. tocopherol/lb. live wgt./day) +(1)] and, in the case of the remaining tissues, X_1 and X_2 were mg. tocopherol/lb. live wgt./day. The regressions were weighted by procedures as discussed in the text.

[b] Percent of the total variation about treatments accounted for by the indicated regressions.

[c] $(B_2/B_1)(100)$.

[d] 95% confidence limits.

rier are in partial agreement with previous work in the chick (Singsen *et al.*, 1955; Bunnell, 1957). It appears probable that the results using tissue concentration of tocopherol as a criterion of availability may not give comparable results to using symptoms characteristic of tocopherol deficiency, such as muscular dystrophy, as is suggested for example, by

the data of Keith and Schneider (1957) with calves and sheep and Hogue *et al.* (1957) with lambs. Both the aforementioned groups of investigators suggested that the incidence of muscular dystrophy may not be closely related to the tocopherol content of the feeds fed. However, in the above mentioned studies with chicks, one using the incidence of encephalomalacia and the other using liver concentration of tocopherol, alfalfa was found to have a lower tocopherol value, approximately one-fourth to one-third of the chemically determined tocopherol content. Considerably more data and more experience are needed before the biological significance of the data contained herein and of the data contained in the above mentioned references can be adequately elucidated.

Histology. Histological studies of the tissues taken from the heart and trapezius muscle failed to reveal any significant alterations. This was probably to be expected, since the basal ration at the levels fed provided an intake between 0.1 and 0.2 mg. of tocopherol per lb. live weight per day based on previous calculations (Rousseau *et al.*, 1957). The experiment was of only short duration, 12-wk., and no so-called "stress" agents such as cod liver oil or other potent sources of unsaturated fats were contained in the basal ration.

Summary

Forty Holstein male calves were fed to 63 days of age on a limited whole milk, limited calf starter and *ad libitum* hay regimen. Each calf was then fed a vitamin A depletion ration at an intake to provide an anticipated 7-day increase in live weight of 10 lb. plus sufficient vitamin A to provide ample storage. On the 92nd day of age, each calf was fed in addition to the above basal ration plus vitamin A, one of five levels of tocopherol from a dry carrier, 0.000, 0.128, 0.256, 0.512 or 1.024 mg. of free tocopherol per lb. of live weight daily, or one of five levels of tocopherol from artificially dehydrated alfalfa leaf meal, 0.000, 0.125, 0.250, 0.500, and 1.000 mg. of free tocopherol. On the 176th day of age each calf was slaughtered. Based on slope-ratio biological assays, the chemically determined total tocopherol in artificially dehydrated alfalfa leaf meal was found to be 102% as available to the calf as tocopherol from the dry carrier using plasma tocopherol as the criterion. Similar values for liver, heart, trapezius muscle and perinephric fat, based on tissue concentrations of tocopherol at slaughter, were on a wet basis, 77%, 99%, 116%, and 65%, respectively, and on a lipid basis, 80%, 96%, 115%, and 65%, respectively. Averaged unweighted percentages were 92% on a wet basis and 89% on a lipid basis. Considering these percentage availability values *in toto,* it was tentatively suggested that the chemically determined total tocopherol in artificially dehydrated alfalfa leaf meal was not completely available to the dairy calf.

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GRAIN SORGHUM FOR GROWING-FINISHING SWINE[1]

E. R. PEO, JR. AND D. B. HUDMAN

Nebraska Agricultural Experiment Station,[2] *Lincoln*

CONSIDERABLE research has been conducted on the feeding value of grain sorghums for swine. Baker and Reinmiller (1939) observed that for the production of gains, grain sorghums were equal to or better than corn but considerable more feed was required to produce a pound of gain with the sorghums. Loeffel (1957) summarized several Nebraska swine experiments and observed that grain sorghum and corn produced approximately the same average daily gain, but, in general, 10 to 20% more feed was required per pound of gain with the grain sorghum. Similar results have been reported by Aubel (1950, 1954, 1955, 1956) and Hillier *et al.* (1954).

For the most part, only the standard varieties of grain sorghums have been studied in swine rations. Also, the rations used in the early studies were not as highly fortified as are our present ones. For these reasons experiments were conducted to compare the feeding value of certain standard and hybrid varieties of grain sorghum with corn when fed either as the entire, or as a part of, the grain portion of well-fortified swine rations.

Experimental Procedure

Experiment 1. Two hundred and twenty-four purebred Yorkshire, purebred Hampshire and Yorkshire-Hampshire crossbred pigs which averaged 56.5 lb. live weight were alloted at random to four replications of eight experimental treatments on the basis of breeding and weight outcome groups. The pigs were housed in concrete-floored pens that were equipped with self-feeders and waterers. The pens were cleaned daily and straw was used for bedding.

The experimental ration treatments were as follows: Corn. (1) 8.5% protein corn; (2) 11.0% protein corn. Grain sorghum varieties. (3) Martin; (4) Reliance; (5) Hybrid RS 590; (6) Hybrid RS 650; (7) Hybrid RS 501; (8) Hybrid RS 610. A brief agronomic description of the hybrid grain sorghums is as follows:

Hybrid RS 590 is a cross between *ms* (male sterile) Combine Kafir-60 ×Redbine 60. It is one to two days earlier than Martin (Martin blooms

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in approximately 67 days). *Hybrid RS 650* is a cross between *ms* Combine Kafir-60×Plainsman. It heads in about the same number of days as Martin. *Hybrid RS 501* is a cross between *ms* Combine Kafir-60× Norghum. This is the earliest hybrid, maturing about 2 or 3 days later than Reliance (Reliance blooms in approximately 55 days). *Hybrid RS 610* is a cross between *ms* Combine Kafir-60×Combine 7078. It is approximately 3 days earlier in heading than Martin.

Since standard corn is usually lower in protein than milo, one treatment consisted of a medium protein corn which contained about the same amount of protein as milo (calculated as 11%). The corn and milo were coarsely

TABLE 1. COMPOSITION OF EXPERIMENT RATIONS
(Experiment 1)

Ration designation[a]	Regular corn	Medium corn	Milo
Ingredients, lb.			
Ground yellow corn	79.4	....	....
Ground yellow corn (medium protein)	....	85.4	....
Ground milo (all varieties)	....	....	85.7
50% meat and bone meal	2.5	2.5	2.5
17% dehydrated alfalfa meal	2.5	2.5	2.5
44% solvent soybean oil meal	12.6	6.6	6.5
Ground limestone	0.5	0.5	0.7
Steamed bone meal	0.9	0.9	0.5
Salt (iodized)	0.5	0.5	0.5
Trace minerals (high zinc, swine)	0.1	0.1	0.1
Vitamin-antibiotic premix[b]	1.0	1.0	1.0

[a] Calculated protein content, 14%.
[b] Contributed the following amounts of vitamins and antibiotics per pound of complete ration: Vit. D_2, 300 I.U.; riboflavin, 2.2 mg.; niacin, 6.0 mg.; calcium pantothenate 3.0 mg.; choline chloride, 110 mg.; Vit. B_{12} 5 mcg.; and antibiotics, 5 mg.

ground by a burr mill and were fed in completely mixed rations. The composition of these rations is presented in table 1. The experiment was terminated after 49 days (average final pig weight, 128.8 lb.) because of a limited supply of certain of the hybrid varieties.

Experiment 2. Seventy-five crossbred pigs (Hampshire x Yorkshire or the reciprocal cross) which averaged 47.7 lb. live weight were allotted at random by weight outcome groups to three replications of five experimental treatments. Housing and management were essentially the same as previously described for the first experiment.

The experimental rations and their respective compositions are presented in table 2. As indicated by the ration treatments, one-third, two-thirds and all of the corn in the basal ration was replaced with grain sorghum (Martin variety). In these particular treatments all other ration components were held at a constant level. The basal ration (all corn) calculated 14% protein whereas in the other rations, where corn was replaced with grain sorghum, the protein content increased progressively to a total

of 16% for the ration (treatment 4) in which all of the corn was replaced with grain sorghum on a pound for pound basis. In addition, one experimental treatment (treatment 5) consisted of an all grain sorghum ration calculated to contain 14% protein for a comparison with the 14% all-corn ration and the other ration treatments. The corn and grain sorghum were ground with a burr mill and fed in completely mixed rations. The experiment was terminated after 70 days.

TABLE 2. COMPOSITION OF EXPERIMENTAL RATIONS
(Experiment 2)

Treatment	1	2	3	4	5
Ration designation[a]	All corn	⅔ corn ⅓ milo	⅓ corn ⅔ milo	All milo	All milo
Ingredients, lb.					
Ground yellow corn	79.4	53.2	26.2	....	....
Ground milo (Martin)	....	26.2	53.2	79.4	85.7
44% solvent soybean oil meal	12.6	12.6	12.6	12.6	6.5
50% meat and bone meal	2.5	2.5	2.5	2.5	2.5
17% dehydrated alfalfa meal	2.5	2.5	2.5	2.5	2.5
Ground limestone	0.5	0.5	0.5	0.5	0.7
Steamed bone meal	0.9	0.9	0.9	0.9	0.5
Salt (iodized)	0.5	0.5	0.5	0.5	0.5
Trace minerals (high zinc, swine)	0.1	0.1	0.1	0.1	0.1
Vitamin-antibiotic premix[b]	1.0	1.0	1.0	1.0	1.0

[a] Calculated protein content 14.0, 14.6, 15.3, 16.0 and 14.0%, respectively.
[b] Contributed the following amounts of vitamins and antibiotics per pound of complete ration: Vit. D_3, 300 I.U.; riboflavin, 2.2 mg.; niacin, 6.0 mg.; calcium pantothenate, 3.0 mg.; choline chloride, 110 mg.; Vit. B_{12}, 5 mcg; and antibiotics, 5 mg.

In both experiments, pig weight and feed data were collected at two-week intervals. The Q-test (Snedecor, 1956) was used to test for significance between treatment means. All statements concerning statistical significance are at a probability level of 5% or less.

Results and Discussion

Experiment 1. The results of this experiment are presented in table 3. The greatest average daily gain was made by pigs fed the ration of regular (8.5%) protein corn. However, this gain was not significantly different from the average daily gains made by pigs fed Martin, Reliance, Hybrid RS 590, Hybrid RS 650 or Hybrid RS 501 grain sorghum rations. The average daily gains made by pigs fed medium (11%) protein corn and Hybrid RS 610 were significantly less than those made by pigs fed the other rations.

The poor performance of pigs fed medium protein corn may have been caused by either or both of the following factors. First, Frey (1951) has shown that when the protein content of corn is increased genetically, zein

becomes an increasing proportion of the total protein. Since apparently all the proteins (and consequently amino acids) in corn are not increased uniformly, the possibility for the occurrence of an amino acid imbalance and/or deficiency in swine rations may increase through the use of medium protein corn. Second, the medium protein corn was 2 years old and, therefore, perhaps was not as digestible as the new-crop regular (8.5%) protein corn; 0.15 lb. more feed was consumed per day by the pigs fed medium protein corn.

TABLE 3. COMPARISON OF CORN AND CERTAIN VARIETIES OF GRAIN SORGHUM FOR GROWING-FINISHING SWINE [a]

(Experiment 1)

Treatment	1	7	8	6	4	3	2	5
Ration designation	Regular corn	Hybrid RS 501	Hybrid RS 610	Hybrid RS 650	Reliance	Martin	Med. protein corn	Hybrid RS 590
Pens per treatment, no.	4	4	4	4	4	4	4	4
Pigs per pen, no.	7	7	7	7	7	7	7	7
Av. initial wt., lb.	56.4	56.3	56.4	56.2	56.5	56.4	56.2	56.1
Av. final wt., lb.	131.9	131.5	123.9	129.1	128.9	130.6	124.7	129.6
Av. total gain, lb.	75.5	75.2	67.5	72.9	72.4	74.2	68.5	73.5
Av. daily gain, lb.	1.54	1.53	1.38[b]	1.49	1.48	1.51	1.40[b]	1.50
Feed per lb. gain, lb.[c]	3.26	3.47	3.51	3.56	3.61	3.67	3.69	3.93

[a] Duration of test, 49 days. One pig from each of treatments 3 and 4 removed because of hernia.
[b] Mean difference significant at P=0.05 or less.
[c] Means underlined by the same line are not significantly different at P=0.05 or less.

The poor gain performance of the pigs fed Hybrid RS 610 can be attributed (in part at least) to a reduced feed intake. Aubel (1951) and Hillier and associates (1954) observed considerable differences in the palatability of several grain sorghum varieties. A difference in tannic acid content may account for such difference in palatability. Apparently Hybrid RS 610 was less palatable than the other grain sorghums (if feed consumption is used as an index of palatability) since the pigs ate less of this variety.

Pigs fed regular protein corn required significantly less feed per pound of gain than those fed the other rations (table 3). Significantly less feed was required per pound of gain with rations containing Hybrids RS 501, 610, 650 and Reliance than with Martin, medium protein corn and Hybrid RS 590. The pigs fed Hybrid RS 590 required the most feed (3.93 lb.) per pound of gain. The difference between this value and the values for the other ration treatments was significant.

Experiment 2. The results of this experiment are presented in table 4. The pigs on the grain ratio of ⅓ corn and ⅔ milo (treatment 3) made an average daily gain of 1.86 lb. This gain was significantly greater than the gains made by pigs subjected to the other ration treatments except treatment 1, the all-corn ration.

Pigs on ration treatment 1 gained 1.80 lb. per day, which was not significantly different from the gain by pigs on treatments 2 and 4. The least gain was made by pigs on treatment 5 (all milo, 14% protein), this gain being significantly less than that made by pigs on ration treatments 1 and 3.

The pigs fed an all-corn ration (grain portion) required the least feed to produce a pound of gain. However, only 0.05 lb. more feed was required to produce a pound of gain with treatment 3, the ration which had a grain ratio of ⅓ corn and ⅔ milo. This difference was not statistically significant. The pigs fed these rations required significantly less feed per pound of gain than pigs on ration treatment 5.

TABLE 4. RATIOS OF CORN AND GRAIN SORGHUM FOR GROWING-FINISHING SWINE [a]

(Experiment 2)

Treatment	3	1	2	4	5
Ration designation	⅓ corn ⅔ milo	All corn	⅔ corn ⅓ milo	All milo	All milo
Calculated protein, %	15.3	14.0	14.6	16.0	14.0
Pens per treatment, no.	3	3	3	3	3
Pigs per pen, no.	5	5	5	5	5
Av. initial wt., lb.	47.9	47.5	47.7	47.6	47.6
Av. final wt., lb.	178.1	173.6	171.3	170.8	167.8
Av. total gain, lb.	130.2	126.1	123.6	123.2	120.2
Av. daily gain, lb. [b]	1.86	1.80	1.77	1.76	1.72
Feed per lb. gain, lb. [b]	3.63	3.58	3.81	3.78	3.97

[a] Duration of test, 70 days.
[b] Means underlined by the same line are not significantly different at P=0.05 or less.

Although the results of the two all-milo treatments were not significantly different, there was a tendency for the 16% protein ration (treatment 4) to produce greater and more economical gains than the 14% protein ration (treatment 5). This would appear to indicate that the amount and kind of protein supplement is more critical in a sorghum ration than in a corn ration. That is, when formulating swine rations with grain sorghums, the higher protein content of grain sorghum, as compared with corn, may increase the opportunity for an amino acid deficiency or imbalance because less total supplemental protein (animal or plant) is required to provide a given protein level. This has been shown clearly by the research of Hillier *et al.* (1954, 1955, 1956) and Hillier (1957) who reported that lysine is probably the first limiting amino acid in milo rations since milo is lower than corn in lysine and there is less supplemental protein as a source of lysine in milo rations.

Summary

Two hundred and twenty-four pigs were used to compare corn (8.5% and 11% protein) with grain sorghums for growing-finishing swine. Although the greatest average daily gain was made by the pigs fed 8.5% protein corn, the difference between those fed corn and certain of the grain sorghums was not significant. The smallest gains were made by pigs fed 11% protein corn and the grain sorghum Hybrid RS 610. In general, it required approximately 0.2 to 0.7 lb. more feed to produce a pound of gain with grain sorghums than with corn, depending upon the variety fed.

When corn and milo were fed alone or in combination at different proportions the greatest average daily gains were made by the pigs fed a ration composed of ⅓ corn and ⅔ milo (grain portion). The least gains were made by those fed an all milo, 14% protein ration. Gains on the all-milo ration increased as the level of protein was increased from 14% to 16%, but were still significantly less than the gains made on a combination of ⅓ corn and ⅔ milo.

Pigs fed an all-corn ration required the least feed per pound of gain; but, only 0.05 lb. more feed per pound gain was required by pigs fed the ⅓ corn–⅔ milo ration. Significantly more feed was required per pound of gain with an all-milo, 14% protein ration.

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HIGH OIL-BEARING SEEDS AND TALLOW-SOYBEAN OIL MEAL IN LAMB FATTENING RATIONS [1]

R. M. JORDAN, H. G. CROOM AND HARLEY HANKE

University of Minnesota [2]

THE use of waste animal fats as components of livestock and poultry feed has been suggested as a means of utilizing a part of the 700 million pounds of excess fat produced annually. There is a lack of information as to whether added fat contributes anything over and above its caloric content, what effect it has on carcass quality and the amount that can be most advantageously incorporated into feeds. Hale *et al.* (1954) fed 5% and 9% added fat in a practical lamb fattening ration. At the 5% level, feed consumption and rate of gain was reduced. Carcass quality was not adversely affected. At the 9% level, feed consumption was very materially reduced as well as rate of gain and carcass quality. Hale *et al.* (1956) reported that 3% fat added to lamb fattening rations had no adverse effect on feed consumption. Lambs so fed actually ate more and gained slightly faster. Feed efficiency was not affected and the authors concluded that the fat was not highly utilized by the lambs. Brooks *et al.* (1954) used an artificial rumen technique in studying the effect of added fat on cellulose digestion. They reported a 40% to 94% reduction in cellulose digestion when corn oil was added to the media. These workers observed a significant reduction in cellulose digestion and lower protein digestion in sheep following the addition of 32 or 64 gm. of corn oil to a basal cotton-seed hull and casein ration. Erwin *et al.* (1956) reported that the addition of 7% fat significantly increased rates of gain of steers fed pellets containing alfalfa. The same level of fat significantly reduced rates of gain of steers consuming wheat straw rations. Willey *et al.* (1952) reported that steers fed fattening rations containing about 7.5% total fat, consumed approximately 2 to 3 lb. less feed daily than steers fed rations containing 2.8% to 2.9% fat. However, feed efficiency was improved by the higher levels of fat.

The objectives of this experiment were to study the effect on rate of gain, feed consumption, feed efficiency, carcass grade and the color and firmness of fat of feeding lambs rations containing high levels of fat as supplied either by the high oil-bearing seeds, soybeans and flax, or by adding animal fat to the protein supplement.

Procedure

Good quality western or native feeder lambs were sheared, individually weighed, and allotted to treatments from outcome groups on the basis

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of weight. At the time of slaughter, individual carcass grade, carcass color, carcass firmness, and dressing percentage by groups were obtained. In the first experiment consisting of four separate trials, the lambs were hand-fed a fattening ration of shelled corn, non-legume hay and a protein supplement. The control lambs were fed solvent-extracted soybean oil meal as the protein supplement, and the treated groups were fed solvent-extracted soybean oil meal with 10% inedible stabilized white tallow added.

In the second experiment, the effects of feeding high-oil seeds, soybeans or flax, to fattening lambs were studied in three separate trials. High quality alfalfa hay was fed to all of the lambs. Soybean oil meal was fed to the control lambs, but no additional protein supplement was fed to the lambs receiving the high-oil seed. The following concentrates were fed: Trial I, lot 1, shelled corn, soybean oil meal; lot 2, equal parts of shelled corn and whole soybeans; lot 3, whole soybeans. Trial II, lot 1, shelled corn and soybean oil meal; lot 2, equal parts of shelled corn and whole soybeans; lot 3, ¾ corn and ¼ whole soybeans. Trial III, lot 1, shelled corn plus soybean oil meal; lot 2, ¾ corn and ¼ flax seed; lot 3, equal parts of shelled corn and whole soybeans; lot 4, ¾ shelled corn and ¼ whole soybeans.

Analysis of variance (Snedecor, 1946) was the method used for the statistical analysis of the data.

Results

Soybean Oil Meal Plus Tallow. The results of the four trials, in which soybean oil meal with added stabilized tallow was compared with solvent-extracted soybean oil meal, are presented in table 1. The addition of 10% stabilized tallow to the soybean oil meal supplement had no statistically significant effect on the rates of gain of fattening lambs. However, in three of the four trials, lambs fed the tallow-fortified soybean oil meal gained somewhat more rapidly than the lambs fed solvent extracted soybean oil meal. In the first trial, feed consumption was somewhat lower in the lot receiving the tallow-soybean oil meal. This lower feed consumption in Trial I may account for the slightly slower gains made by that lot. In the other three trials, no difficulty was experienced in keeping the lambs on full feed and the average daily feed consumption was similar to their respective controls. Feed efficiency was not affected materially by treatment.

Carcass grade and yield data were obtained in only three of the four trials. There was no significant difference between treatments in carcass grade. However, in two of the three trials, where carcass data were obtained, lambs fed tallow-fortified soybean oil meal graded slightly lower and had slightly lower carcass yields than the control lambs. There was no evidence of softness of the fat or of bronze color of the carcasses of any of the lambs.

High-Oil Seeds. The data pertaining to the lambs fed high-oil seeds as a large part of their concentrate ration are presented in table 2. The feeding of whole soybeans to fattening lambs reduced the rate of gain significantly ($P<.01$). The lambs were difficult to keep on feed and consumed decidedly less concentrate and hay during the entire feeding period. Because of their poor gains, over four times as much feed was required to produce a pound of gain as was required by the control lambs. As might be expected from such low gains, the carcasses were under-finished and low-

TABLE 1. THE EFFECT OF FEEDING TALLOW-SOYBEAN OIL MEAL SUPPLEMENTS TO FATTENING LAMBS

Trail	I		II		III		IV	
Lot	1	2	1	2	1	2	1	2
Treatment [a]	S.O.M.	Tallow	S.O.M.	Tallow	S.O.M.	Tallow	S.O.M.	Tallow
Number of lambs	10	10	10	10	15	15	15	15
No. days fed	49	49	98	98	84	84	56	56
Av. initial wt., lb.	86.2	87.2	66.0	66.1	57.7	57.2	73.3	72.5
Av. gain, lb.	15.5	14.2	40.4	42.6	33.2	35.1	22.5	25.3
Av. daily gain, lb.	0.32	0.29	0.41	0.45	0.40	0.42	0.40	0.45
Av. daily feed consumption								
Grain, lb.	1.76	1.67	1.56	1.53	1.50	1.50	1.39	1.41
Hay, lb.	1.39	1.38	1.16	1.17	1.27	1.19	1.19	1.15
Protein supplement, lb.	0.30	0.30	0.30	0.30	0.31	0.31	0.25	0.25
Feed per 100 lb. gain, lb.								
Grain	556	573	378	353	379	358	349	311
Hay	440	471	282	268	323	286	298	255
Protein supplement	95	103	73	69	80	76	64	56
Carcass grade [b]	5.9	6.4	6.2	5.9	6.9	6.5	...	...
Yield	53.4	53.9	50.1	49.4	52.0	49.1	...	...

[a] In each trial Lot 1 was the control and Lot 2 the experimental.
[b] Carcass grade code: Low choice, 7; High good, 6; Average good, 5.

yielding. However, this high level of oil feeding did not produce soft, oily or bronze colored carcasses.

When whole soybeans constituted 50% of the concentrate ration (lot 2 in Trial I and II, lot 3 in Trial III) the average daily gain was significantly reduced in Trials I and III and approached significance at the 5% level in Trial II. In each of the three trials, lambs fed a concentrate of equal parts of corn and whole soybeans consumed less concentrate than the control lambs. The high oil content of the concentrate fed reduced the amount of hay consumed in Trial I but not in Trials II and III. The lower concentrate consumption of the lots receiving equal parts of whole soybeans and corn appears to be a major factor in causing lower gains. Since the concentrate consumption was not reduced when the "grain" ration contained only 25% raw soybeans it was concluded that the reduction in concentrate consumption when 50% raw soybeans were fed was due to the high level of oil in the ration and not to any component of the raw soybeans. Hale *et al.* (1954) and Willey *et al.* (1952) reported similar reductions in feed intake when the ration contained 7–9% fat.

TABLE 2. THE EFFECT OF FEEDING HIGH-OIL SEEDS TO FATTENING LAMBS

Trial	I			II			III			
Lot	1	2	3	1	2	3	1	2	3	4
Number of lambs	10	10	10	10	10	10	12	12	12	12
No. days fed	49	49	49	77	77	77	84	84	84	84
Av. initial wt., lb.	88.1	84.7	87.4	74.6	71.3	69.4	68.6	68.6	68.6	68.5
Av. gain, lb.	17.3	6.0	2.9	31.3	27.7	29.7	45.1	42.7	36.1	44.3
Av. daily gain, lb.	0.35	0.12**	0.06**	0.41	0.36	0.38	0.54	0.51	0.43**	0.53
Calculated fat content of grain rations, %	3.9	10.9	18.0	3.9	10.9	7.4	3.9	11.9	10.9	7.4
Av. daily feed consumption										
Grain, lb.	1.78	1.39	1.18	1.64	1.44	1.63	1.65	1.75	1.51	1.74
Hay, lb.	1.47	1.30	1.24	1.15	1.15	1.19	1.68	1.63	1.67	1.71
Protein supplement, lb.	.20	....	....	.10	....	....	.10	....	....	....
Feed per 100 lb. gain, lb.										
Grain	505	1133	2000	404	400	425	307	344	351	330
Hay	417	1062	2100	283	319	309	313	321	388	324
Protein supplement	57	....	....	24	...	...	19	...	...	...
Carcass grade[a]	6.3	5.9	5.8	6.3	5.8	5.5	6.5	6.7	5.4	6.4
Yield	54.8	52.1	51.5	52.3	51.1	54.1	54.1	54.2	53.0	54.6

Trial I: Lot 1, control; Lot 2, ½ corn, ½ soybeans; Lot 3, soybeans.
Trial II: Lot 1, control; Lot 2, ½ corn, ½ soybeans; Lot 3, ¾ corn, ¼ soybeans.
Trial III: Lot 1, control; Lot 2, ¾ corn, ¼ flaxseed; Lot 3, ½ corn, ½ soybeans; Lot 4, ¾ corn, ¼ soybeans.
** Significant difference at the 1.0% level of probability.
[a] Carcass grade code: Low choice, 7; High good, 6; Average good, 5.

The feeding of a ration consisting of 75% corn and 25% whole soybeans (lot 3 in Trial II and lot 4 in Trial III) did not significantly affect the average daily gain in either trial. The grain and hay consumption was not affected by the inclusion of this level of whole soy beans in the ration.

The design of this experiment was such that a statistical analysis of the data on feed efficiency was impossible. In both Trials II and III the control lambs and those fed a concentrate containing 25% soybeans consumed similar amounts of total concentrates. The high fat content (7.4%) and therefore the high caloric content of the concentrate containing soybeans did not result in greater feed efficiency. This is in agreement with the work reported by Hale *et al.* (1956).

The carcass grades and yields of the lambs receiving equal parts of corn and whole soybeans or 75% corn and 25% whole soybeans largely reflect the differences in rate of gain and were somewhat lower than those of the control lambs.

In trial III, the lambs in lot 2 received a "grain" ration consisting of 25% flaxseed and 75% shelled corn. Average gains were slightly lower for this lot than for either the control or the lot receiving the ration consisting of 25% whole soybeans and 75% corn. However, these differences in rate of gain were not significant.

There was equal concentrate (grain and protein supplement) consumption by the control lambs and the lambs fed 25% flaxseed. However, feed efficiency was 11% higher in the control lot. These data indicate that when the grain ration contains as much as 7% fat the efficiency of feed utilization by fattening lambs is reduced.

Lambs fed whole flaxseed as 25% of the concentrate ration produced carcasses that had more bloom and graded higher than the lambs fed a concentrate ration containing 25% soybeans. No differences in color or softness of the fat could be detected between the control lambs and any of the lambs that had received grain rations containing a relatively high percentage of fat. This observation indicates that the fat content of the ration is not the main cause of soft, bronze colored lamb carcasses that constantly plague the packing industry.

Summary

Four lamb fattening trials were conducted to determine the effect on rate of gain, feed consumption and efficiency of feed utilization of adding 10% stabilized tallow to soybean oil meal. Rate of gain was not significantly affected. However, in three of the four trials the lambs fed soybean oil meal with added fat gained slightly faster and required somewhat less feed per pound of gain than the control lambs.

Fattening lambs were fed "grain" rations consisting of whole soybeans, equal parts of whole soybeans and corn, 25% soybeans and 75% corn, and 25% flaxseed and 75% corn. Rates of gain and feed consumption

were materially reduced when the "grain" ration consisted of either whole soybeans or equal parts of whole soybeans and corn but not when it contained 25% soybeans or 25% flaxseed. It was concluded that the lower gains made by lambs fed grain rations containing 50% or more of whole soybeans were primarily due to reduced feed intake caused by the high oil content of the rations. However, when 25% soybeans or flaxseed were fed, with no reduction in feed intake, the efficiency of feed utilization was reduced. No soft and/or bronze colored lamb carcasses were produced by the feeding of grain rations containing high levels of fat.

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RELATIONSHIP OF LIVE MEASUREMENTS AND CARCASS CHARACTERISTICS OF SWINE [1]

L. A. HOLLAND [2] AND L. N. HAZEL

Iowa State College,[3] Ames

EARLY studies of the relationship between live animal characteristics and carcass characteristics of swine included age at slaughter, rate of gain, visual scores, and body measurements. Generally, measurements of these characteristics were only slightly related to measurements of carcass characteristics. The probe measurements of backfat thickness on live pigs reported by Hazel and Kline (1952) provide a fairly satisfactory method of measuring fatness. However, an accurate method of measuring muscle thickness directly on live pigs would be of considerable value. So far as is known, this is the first effort to develop such a method. The purpose of this study was to measure muscle thickness and fat thickness over the supraspinous fossa and over the ilium on live pigs and to compare these measurements with other live animal measurements and carcass measurements as indicators of carcass characteristics.

Methods and Data

Measurements were taken during the fall of 1955 on 105 purebred barrows from the swine project maintained cooperatively by the Iowa Agricultural Experiment Station and the Regional Swine Breeding Laboratory. The pigs were a sample of purebred barrows by each of several sires within the seven breeds maintained at the Napier swine breeding farm. The pigs were raised on alfalfa pasture and received corn and protein supplement supplied free-choice in self-feeders. Each week those which had reached a minimum weight of 210 lb. were slaughtered. The pigs were deprived of feed but allowed access to water for 24 hours before slaughter.

Live Animal Measurements. The pigs were scored for degree of finish by the junior author either when taken off feed or at the end of the shrink period. The range in scores was 5 to 9, score 5 designating slightly underfinished, and 9 designating highly overfinished barrows.

All other animal measurements were taken immediately before slaughter by the senior author. Each pig was restrained by looping one end of a hog catcher around the mandible and tying the other end of the catcher to a ring in the wall of the meats laboratory. The pig, resisting the catcher, strained backward with the top of his head about level with the top line

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[2] Present address: Department of Animal Husbandry, Kansas State College, Manhattan, Kansas.

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of his body. Jowl circumference was measured by passing a steel tape around the eyes, perpendicularly to the floor. Body length was measured with the tape along the top line from between the ears to the base of the tail. Flank circumference was measured by passing the tape around the body immediately in front of the hams. Middle circumference was taken at a point judged midway between the front and rear legs. Chest circumference was measured immediately behind the shoulder.

Probe measurements of backfat thickness were made with a metal ruler as described by Hazel and Kline (1952). The probing sites were behind the shoulder, at the middle of the back, and at the middle of the loin, in each case being 1½ to 2 inches off the midline of the body.

The probing site over the shaft of the ilium was determined by visual observation. Fat depth was measured with a metal ruler. The combined thickness of fat and lean at this site was measured with a pick probe. The pick probe was an ordinary six-inch ice pick reduced to a maximum diameter of 0.076 inch. The pick probe was inserted through the incision made in measuring thickness of fat and pushed through the tissue until the point touched the shaft of the ilium. Then a cork on the pick was pushed to the skin surface; the pick probe was withdrawn, and depth of penetration was measured with a ruler in millimeters from the tip of the probe to the bottom of the cork. The fat depth was subtracted from this measurement to obtain the thickness of lean which was recorded as ilium lean.

The probing site over the supraspinous fossa was about one inch anterior to the tuber spinae which was located by palpation. The thickness of fat, measured with the metal ruler, was subtracted from the pick probe reading made from the skin surface to the underlying bone to obtain the thickness of lean which was recorded as scapula lean.

Carcass Measurements. The pigs were slaughtered packer style except that the leaf fat and ham facings were loosened but were left attached to the carcass. The carcasses were split immediately after slaughter and were chilled for either 48 or 72 hours at a temperature of approximately 34° F.

Carcass length was the average of measurements on the right and left side of the carcass from the first rib to the anterior edge of the aitch bone.

Thickness of backfat was measured on both sides of the split carcass at the first, seventh, and last thoracic vertebrae, and at the last lumbar vertebra. The average of these measurements was designated as average of eight carcass backfat measurements. The average of the measurements excluding the measurements at the seventh thoracic vertebrae was designated as average of six carcass backfat measurements.

Both sides of the carcass were cut as described in the fifth annual Reciprocal Meat Conference Proceedings (1952). Before the fat was trimmed from the loins, tracings were made of the longissimus dorsi at the tenth and last rib on both loins. Two planimeter readings were made of each tracing and the four readings at each rib were averaged.

Percent lean cuts was computed by dividing the sum of the weights of the closely trimmed hams, loins, Boston butts, and picnics by the weight of the chilled carcass and multiplying by 100.

Percent fat cuts was computed by dividing the sum of the weights of the leaf fat, backfat, and clear plate by chilled carcass weight and multiplying by 100.

Results and Discussion

The means and standard deviations are shown in table 1. Degrees of freedom for the standard deviations were 104 for total and 98 for intrabreed. Differences among the breed means, not listed herein, were statistically highly significant for all the variables except age, shrunk live weight, and jowl circumference. Therefore, correlations were computed intrabreed

TABLE 1. MEANS AND STANDARD DEVIATIONS OF VARIABLES

Variable	Mean	Standard deviation	
		Total	Intrabreed
Live animal measurements			
Age (days)	200.7	20.3	19.5
Shrunk live weight, lb.	207.8	5.7	5.6
Condition score	6.98	1.14	.84
Body length, cm.	109.0	4.42	3.67
Circumference, cm.			
Flank	110.7	4.01	3.46
Middle	115.4	3.55	2.54
Chest	109.6	3.62	2.96
Jowl	60.6	3.62	3.41
Backfat probes, cm.			
Behind shoulder	4.32	.71	.57
Middle back	3.72	.54	.45
Middle loin	3.09	.50	.39
Av. 3 probes	3.71	.51	.39
Ilium, cm.			
Fat	3.70	.63	.49
Lean	4.56	.70	.63
Scapula, cm.			
Fat	2.35	.56	.47
Lean	3.15	.70	.69
Carcass measurements			
Carcass length, cm.	74.37	2.71	1.76
Carcass backfat thickness, cm.			
Average of eight	4.20	.51	.42
Average of six	4.23	.48	.43
Area of loin, sq. in.			
10th rib	3.28	.46	.35
Last rib	3.47	.51	.36
Percent lean cuts	46.48	2.93	2.20
Percent fat cuts	17.38	2.38	1.59

to remove differences among the breed means. The total correlations contain differences among the breed means and, as shown in tables 2 and 3, are usually larger in absolute magnitude than the intrabreed correlations. Standard errors are about 0.10 for correlations under 0.50 and are a bit smaller for larger correlations. The intrabreed correlations are believed to be more useful indicators of the relationship between variables within a breed.

TABLE 2. SIMPLE CORRELATIONS BETWEEN VARIABLES AND PERCENT LEAN CUTS AND PERCENT FAT CUTS

Items	Percent lean cuts		Percent fat cuts	
	Total	Intrabreed	Total	Intrabreed
Live animal measurements				
Age	0.12	0.03	—.17	0.00
Condition score	—.56	—.25	.58	.22
Body length	.20	.03	—.33	.06
Circumference				
Flank	—.34	—.12	.37	.20
Middle	—.60	—.32	.69	.43
Chest	—.49	—.29	.54	.32
Jowl	.16	.20	—.16	—.15
Backfat probes				
Behind shoulder	—.60	—.43	.70	.57
Middle back	—.72	—.60	.81	.72
Middle loin	—.71	—.61	.78	.71
Av. of 3 probes	—.78	—.64	.87	.80
Ilium				
Fat	—.68	—.57	.64	.46
Lean	.26	.18	—.23	—.03
Scapula				
Fat	—.49	—.34	.54	.49
Lean	—.10	.00	.03	—.06
Carcass measurements				
Length	.33	.25	—.33	—.28
Backfat thickness				
Average of eight	—.72	—.59	.81	.71
Average of six	—.65	—.56	.73	.66
Area of loin				
10th rib	.57	.42	—.50	—.33
Last rib	.58	.43	—.50	—.37

The average of three backfat probes was the most accurate single indicator of percent lean cuts and percent fat cuts among all measurements on live pigs (table 2). It was also more accurate than backfat measurements taken on the carcass. Hazel and Kline (1952) reported a correlation between average of four backfat probes and percentage primal cuts slightly larger than the correlation between average of four carcass backfat measurements and percentage primal cuts. Hetzer *et al.* (1956) stated that with

the possible exception of barrows, measurements of backfat thickness on the live hog seem to be as accurate for predicting percentage preferred cuts as are measurements of backfat thickness on the carcass. DePape and Whatley (1956) reported a correlation between the average of six backfat probes and percent primal cuts larger than the correlation between carcass backfat thickness and percent primal cuts. Their correlation between average of six backfat probes and percent lean cuts was lower than their correlation between carcass backfat thickness and percent lean cuts.

TABLE 3. CORRELATIONS OF PROBE MEASUREMENTS AND CARCASS MEASUREMENTS WITH AREA OF LOIN

	Area of loin			
	10th rib		Last rib	
Items	Total	Intrabreed	Total	Intrabreed
Live animal measurements				
Backfat probes				
Behind shoulder	−.70	−.03	−.38	−.16
Middle back	−.42	−.18	−.40	−.18
Middle loin	−.41	−.16	−.41	−.17
Av. of 3 probes	−.44	−.14	−.46	−.20
Ilium				
Fat	−.44	−.25	−.37	−.20
Lean	.36	.32	.18	.14
Scapula				
Fat	−.40	−.18	−.39	−.18
Lean	.11	.22	.04	.14
Carcass measurements				
Length	.13	−.09	.13	.02
Backfat thickness				
Av. of eight	−.31	−.15	−.29	−.10
Av. of six	−.30	−.16	−.24	−.09

The probe behind the shoulder was the poorest location for measuring backfat on the live pig. This was contrary to the finding of Hazel and Kline (1952). However, Zobriskey *et al.* (1954) listed correlations between the probe behind the shoulder and percent lean cuts and percent fat cuts lower than the correlations involving probe at the hip and the probe at the top of the ham. Hetzer *et al.* (1956) reported lower correlations between the probe behind shoulder and percentage preferred cuts and percentage fat cuts than between the two carcass characteristics and probes at the middle of the back and at the middle of the loin on hogs at 225 lb. live weight. The difference in accuracy of measurements at this site in separate studies may be due to differences in probing techniques of the various investigators. Penetration through the trapezius muscle (false lean) in this region on some pigs and failure to penetrate it on other pigs is a probable source of error that would lower the correlation between the

probe measurement and yield of lean cuts, fat cuts, or primal cuts as compared with that which would result from probing consistently to the trapezius or probing consistently through the trapezius. This source of error was checked in a later experiment where the senior author did probe through the trapezius muscle on 16 out of 50 pigs when he believed he was probing only to the muscle.

The correlation between percent lean cuts and ilium lean was a disappointing 0.18 while that with scapula lean was zero. While the errors caused by choice of probing site, angle of penetration, position of animal and measurement of overlying fat might be reduced with additional experience, the errors encountered in this study are thought to be less than would be encountered with any effort at widespread use of the technique. The conclusion seems inevitable that probing through the muscular tissue at these two sites gives measurements which are too inaccurate to increase appreciably the accuracy which is possible from probing through the fat only. But the probe for fat depth over the ilium appears to have real merit as an indicator of leanness since its intrabreed correlation with percent lean cuts was $-.57$. The only other study involving a similar but not identical site was the "top of ham" reported by Hazel and Kline (1953) who gave correlations between this probing site and percent lean cuts and percent fat cuts of $-.65$ and $+.66$, respectively. In that study, as in the present one, the fat probe over the supraspinous fossa was only slightly related to percent lean cuts.

The present results indicate that age at slaughter has little, if any, effect upon percent lean cuts when pigs are fed the same ration and are slaughtered within a reasonably narrow range in live weight. The low intrabreed correlation of 0.03 between age at slaughter and percent lean cuts was far below statistical significance.

The low intrabreed correlations of condition score with percent lean cuts and percent fat cuts were in agreement with the findings by Bratzler and Margerum (1953) and others that visual scores were not closely related with yields of lean or fat cuts.

Measurements of body dimensions had only slight value for predicting percent lean cuts and percent fat cuts. Hetzer *et al.* (1950) listed low correlations between measurements of body dimensions and yield of five cuts. This agrees with the result of the present study where the pigs with larger circumferences of flank, middle, and chest tended to have slightly smaller percent lean cuts and larger percent fat cuts. Surprisingly, the pigs with the larger jowl circumference tended to have larger lean cuts and smaller percent fat cuts. This correlation, however, was not statistically significant. The measurement of jowl circumference was taken because larger jowls generally are believed to indicate greater fatness and, hence, lower percent lean cuts. The measurement may not have measured largeness of jowl as observed on live pigs when they are standing naturally and are not restrained by a catcher.

Correlations of percent lean cuts and percent fat cuts with carcass length, backfat thickness, and area of loin were in general agreement with correlations reported in earlier investigations. Of the carcass measurements, average backfat thickness was the most accurate indicator of the yields of the cuts; area of loin either at the tenth or last rib was next most accurate; and carcass length was the least accurate of the carcass measurements in predicting yields of the cuts.

All intrabreed correlations of probe measurements and carcass measurements with lean areas of loin were low (table 3). Other workers, Hazel and Kline (1952), DePape and Whatley (1956), Price *et al.* (1957), Pearson *et al.* (1957), reported low but generally higher correlations between probe measurements and lean areas of loin than those reported herein (table 3).

The intrabreed correlation between the average of three probe measurements and the average of eight backfat measurements on the carcass was 0.72, in agreement with similar correlations reported by numerous workers.

Application. Probe measurements of backfat thickness can be utilized extensively for selecting swine replacement stock for meatiness because these measurements far exceed other live animal measurements and condition score as indicators of lean and fat in the carcass. The correlations between percent lean cuts and muscle depth measurements by probing at the ilium and scapula are too low to have practical utility.

The correlations given herein are phenotypic. Genetic correlations between the live measurements and carcass characteristics need to be determined to estimate improvement in carcass characteristics when selections are made on the basis of live animal measurements.

One logical alternative to selecting individual pigs for backfat probe is that of slaughtering samples of pigs and selecting the full brothers and sisters of those having the highest percent lean cuts. The genetic consequences of the two alternatives can be compared provided certain logical assumptions are made. Assuming that backfat probe and percent lean cuts are equally heritable, selecting on the basis of backfat probe will yield more rapid improvement when the genetic correlation between backfat probe and percent lean cuts is greater than —.50. Since the multiple correlation between percent lean cuts and probes for fatness (including the ilium probe) was 0.738, the actual genetic correlation may well be considerably higher than the required minimum of —.50. There are good theoretical reasons for expecting the genetic correlation in this case to be higher than the phenotypic correlation. This expectation is substantiated by the estimated genetic correlation of —.91 obtained by Sutherland (1958) between percent lean cuts and the average of 6 backfat probes on each pig.

The alternative of selecting individual pigs for backfat probe has the one disadvantage of being an indirect measure of percent lean cuts but this is apparently not serious since all available evidence indicates that the relationship with percent lean cuts is quite high both genetically and

phenotypically. The other alternative of slaughtering samples of pigs has an equally serious disadvantage since selection again must be practiced indirectly in selecting the brothers and sisters of the sampled pigs. Other disadvantages which seem likely to lower the effectiveness of this method are that the slaughtered pigs may be biased samples of the groups they represent, that the slaughtering reduces the intensity of selection which might otherwise be practiced, and that the information is not immediately available when selection usually will be practiced.

Since percent lean cuts usually is difficult for private breeders to obtain in commercial slaughter plants, swine certification programs have generally

TABLE 4. MULTIPLE CORRELATIONS, COMPUTED INTRABREED, WITH PERCENT LEAN CUTS AS THE DEPENDENT VARIABLES

Independent variables	Multiple correlation
Backfat probes behind shoulder, middle back, middle loin	0.680
Av. of three backfat probes, ilium fat probe	0.738
Carcass length, av. of six backfat measurements, and loin eye area tenth rib	0.667
Carcass length, av. of eight backfat measurements, and loin eye area last rib	0.700
Av. of eight backfat measurements, and loin eye area last rib	0.697

required data on backfat thickness, lean loin area, and body length. As shown in table 4, combinations of these measurements yield slightly lower multiple correlations with percent lean cuts than do the backfat probes where the ilium probe is included. It is evident that most of these considerations involving accuracy, intensity of selection, ease of complete sampling, and availability of data point to backfat probing as being the method of choice for improvement of carcass meatiness under practical conditions.

While it is impossible to standardize completely the conditions under which pigs are grown on a large number of farms, certain principles need to be emphasized where improvement in carcass quality is a primary objective. First, prospective breeding animals should be self-fed or fed to the limits of their respective appetites. Second, they should be fed a high-energy ration consisting primarily of corn with sufficient protein, vitamins and minerals to balance the ration. Third, they should not be forced to exercise. Although data bearing directly on these principles were not present herein, it seems evident that violating these practices actually will hamper genetic improvement because the inherent tendencies of the pigs to fatten will be obscured.

Summary

Muscle thickness and fat thickness over the supraspinous fossa and over the ilium were measured on 105 live barrows and compared with other live measurements and carcass measurements as indicators of carcass characteristics. The average of three backfat probes was the most accurate indicator of percent lean cuts and percent fat cuts among the live animal measurements. These measurements included backfat probes, probe measurements of muscle thickness and fat thickness over the supraspinous fossa and over the ilium, tape measurements of jowl and body dimensions, condition score, and age at slaughter. The average of three backfat probes was also a more accurate indicator of percent lean cuts and percent fat cuts than were the carcass measurements, length, area of loin eye at the tenth rib and at the last rib, and backfat measurements.

Probe measurements appear to be sufficiently accurate and to have numerous other advantages over alternative method so that they should be used widely in selecting for improvement in meatiness.

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EFFECT OF METHODS OF STILBESTROL ADMINISTRATION ON QUALITY FACTORS OF BEEF

MARION SIMONE, M. T. CLEGG AND FLOYD CARROLL

University of California,[1] *Davis*

THE increase in rate of gain and efficiency of food utilization following stilbestrol administration in beef cattle is of great economic importance. Effects of this hormone on beef cattle, with regard to behavior, physiology, fat deposition, carcass composition and residual hormone, have been widely studied (Clegg and Cole, 1954; Andrews *et al.*, 1954; Clegg and Carroll, 1956, Cahill *et al.*, 1956). To a less extent its effect on the factors influencing eating quality has been investigated. Wierbicki *et al.* (1955, 1956) and Simone *et al.* (1955) reported slightly less tender meat from stilbestrol-treated animals than from comparably fed untreated animals; but, differences in tenderness, juiciness and flavor factors, as evaluaed by a sensory panel testing, were not statistically significant. Deans *et al.* (1956) found no significant differences in shear readings between samples of meat from treated and untreated animals. Thus, among the few workers investigating the effect of stilbestrol on eating quality, only trends toward lower quality, especially in tenderness, have been observed.

Impetus has been given recently to comparative studies on the oral and implant methods of hormone administration (Clegg and Carroll, 1957); Kastelic *et al.*, 1956; Perry *et al.*, 1955). An evaluation of the subjective quality factors of the meat as affected by the treatments has been lacking. The present investigation was undertaken to determine whether stilbestrol administered either orally or by implantation influenced the tenderness, juiciness and flavor of beef as evaluated by sensory panel testing.

Experimental

Thirty-two weanling Hereford steers from the University of California range herd were divided into four groups of eight animals each. These animals were of uniform breeding, age, quality and initial weight. The treatment given to the animals in each of the groups was as follows: Group I was given 10 mg. of stilbestrol per head per day orally throughout the experimental period; Group II received a single 15 mg. pellet subcutaneously implanted in the ear at the outset of the study; Group III received no hormone treatment; and Group IV received no hormone treatment and less concentrate in their ration than the other three groups. The purpose of the latter treatment was to produce lower average USDA carcass grade and finish as compared to Groups I, II, and III.

The ration consisted of roughage (67% high quality alfalfa hay and

[1] Departments of Food Technology and Animal Husbandry.

33% grain hay) and a concentrate composed of 60% ground barley, 15% rolled oats, 20% molasses dried beet pulp, and 5% cottonseed meal (43% protein). Groups I, II and III received a total ration containing approximately 60% of the concentrate and 40% roughage. Animals in Group IV received a ration consisting of 15% concentrate and 85% roughage.

Two animals were selected from each group on the basis of comparable finish after a 193-day feeding period and each week thereafter. All of the animals were slaughtered and graded according to standard practice by a USDA grader at a local packing house. The carcasses were aged under normal practice for 11 days before cutting by the University cafeteria meat department under supervision of the experimenters. Densiometric measurements were made on a 7-rib section of the right side. This section was then divided into two 3-rib roasts (6th, 7th, 8th ribs and 9th, 10th, 11th ribs) for sensory tests. The 12th rib was retained for physical measurement of percent bone, fat and lean, and chemical determination of percent moisture and ether extract (marbling) of the lean. Two adjacent three-inch thick roasts from the center toward the posterior of the top round of each animal were also obtained for subjective quality evaluation.

Four matched round and rib roasts from the four groups of animals were evaluated on successive mornings and afternoons, respectively, through the four-week period of testing.

Both cuts were prepared by roasting in uncovered aluminum pans at 300° F. to an internal temperature of 150° F. The *longissimus dorsi* muscle of the rib roasts and the *semi-membranosus* and *adductor* muscles of the top round roasts were used for the testing. These muscles were carved into ¼ in. to ⅜ in. slices and these slices cut into pieces approximately 2 sq. in. in area. The pieces were trimmed of fat, arranged in shallow enamel pans according to muscle location and kept warm throughout the 20- to 30-minute tasting session.

Each of 10 to 12 judges received a set of four carefully matched pieces with respect to muscle location from the four groups. The samples were arranged randomly opposite code numbers on warmed white serving dishes. Evaluations were made by each judge in individual tasting booths equipped with low red illumination to mask any slight color differences among the samples. Tenderness, juiciness and flavor were evaluated by a scoring system using a 7-point scale for each factor, indicating a very desirable rating; 5, desirable; 2, just acceptable.

The panel was selected from a group of 20 members of the Department of Food Technology faculty, staff and graduate students. The criteria of selection were based on discriminating ability and consistency in evaluating quality factors in commercial samples of beef of various USDA grades.

Results and Discussion

In table 1 are listed the USDA carcass grades and composition of the carcasses in each group. Statistically significant differences in fat, lean,

TABLE 1. CARCASS GRADES AND COMPOSITION

Items	Group I Stilbestrol oral	Group II Stilbestrol implant	Group III No stilbestrol	Group IV No stilbestrol, less finish	Statistics "F" ratio	L.S.D.[a] .05	L.S.D.[a] .01
U.S.D.A. slaughter grade, no. of animals							
Choice	6	8	7	0			
Good	2	0	1	3			
Commercial or standard	0	0	0	5			
Composition, %							
Fat[b]	38.3	37.6	38.0	30.3	12.01***	4.6	5.8
Lean[b]	48.2	48.0	47.9	53.2	11.03***	4.1	5.1
Bone[b]	13.5	14.4	14.2	16.5	4.40*	1.3	...
Moisture[c]	73.6	73.4	73.1	74.8	2.48	...	...
Ether extract[c]	10.6	9.9	11.5	5.4	3.26*	5.5	...

[a] Least significant differences between groups at .05 and .01 probability levels.
[b] Physical separation of right 12th, rib cut, average of eight carcasses.
[c] Chemical analysis of right 12th, rib eye muscle, average of eight carcasses.
* Significant at .05 probability level.
*** Significant at .001 probability level.

bone and ether extract as determined by analysis of variance were obtained only between Group IV and each of the other groups. No differences in composition were obtained between the two stilbestrol-treated groups, nor between either of these groups and the untreated Group III. A more detailed discussion of the treatment effects on the composition and densio-

TABLE 2 TENDERNESS, JUICINESS, AND FLAVOR SCORES (AVERAGE SCORES OF EIGHT REPLICATIONS)

Items	Group I Stilbestrol oral	Group II Stilbestrol implant	Group III No stilbestrol	Group IV No stilbestrol, less finish	Statistics "F" ratio	L.S.D. .05	L.S.D. .01
Tenderness							
Round (anterior)	4.46	4.39	4.94	4.42	2.69	....	....
Round (posterior)	3.91	3.73	4.50	3.73	3.57*	0.74	....
Ribs 9–10–11	5.68	5.73	5.81	5.52	0.49	....	....
Ribs 6–7–8	5.72	5.89	5.99	5.64	0.86	....	....
Av. all cuts	4.94	4.93	5.31	4.83	5.77**	0.34	0.42
Juiciness							
Round (anterior)	4.16	4.07	4.53	3.89	2.41	....	....
Round (posterior)	3.99	4.00	4.07	3.61	2.03	....	....
Ribs 9–10–11	4.94	5.17	4.99	4.89	0.52	....	....
Ribs 6–7–8	5.26	5.00	5.44	5.02	2.88	....	....
Av. all cuts	4.59	4.56	4.76	4.35	4.64**	0.30	0.37
Flavor							
Round (anterior)	4.61	4.70	4.59	4.31	1.71	....	....
Round (posterior)	4.47	4.40	4.54	4.19	1.31	....	....
Ribs 9–10–11	5.24	5.36	5.34	4.93	3.09*	0.42	....
Ribs 6–7–8	5.32	5.33	5.37	5.08	1.06	....	....
Av. all cuts	4.91	4.95	4.96	4.63	6.25***	0.24	0.30

* Significant at .05 probability level.
** Significant at .01 probability level.
*** Significant at .001 probability level.

metric measurements of these carcasses has been presented by Clegg and Carroll (1957).

The tenderness, juiciness and flavor scores presented in table 2 were calculated by averaging the mean panel scores for each animal replication. Analysis of variance was applied to the data for determining the significance of differences among the groups. The least significant difference values (LSD) were determined by the Tukey procedure (tabular $q \times \sqrt{\frac{\text{mean square of error}}{\text{no. of replications per group}}}$).

TABLE 3 ANALYSIS OF VARIANCE (GROUPS AND CUTS)

Items	Source of variation	Degrees of freedom	Sums of squares	Mean squares
Tenderness				
	Groups	3	4.2720	1.4720**
	Cuts	3	76.8187	25.6062***
	Groups×cuts	9	1.5230	0.1692
	Error	112	27.6426	0.2468
	Total	127	110.2563	
Juiciness				
	Groups	3	2.6439	0.8813**
	Cuts	3	36.6925	12.2308***
	Groups×cuts	9	1.5409	0.1712
	Error	112	21.2896	0.1901
	Total	127	62.1169	
Flavor				
	Groups	3	2.3818	0.7939***
	Cuts	3	19.3354	6.4451***
	Groups×cuts	9	0.2282	0.0254
	Error	112	14.2318	0.1271
	Total	127	36.1772	

** Significant at .01 probability level.
*** Significant at .001 probability level.

Within any one cut, statistically significant differences in tenderness among the groups occurred only in the posterior top round roast, indicating that the stilbestrol-treated Groups I and II were comparable to Group IV and that these three groups were less tender than the untreated Group III (table 2). Differences in tenderness among the groups in the other cuts were not statistically significant. Trends, however, toward less tender meat from the stilbestrol-treated animals are evident. When the scores of the four cuts were combined, the differences were highly significant. Although the different cuts were not evaluated simultaneously, very highly significant differences in tenderness scores were ascribed to the four cuts (tables 3 and 4). No real differences in tenderness were observed between the two rib-roasts. However, the anterior round roast was rated significantly more

tender than the posterior round cut. This same difference in tenderness within the top round has been objectively measured by Paul and Bratzler (1955). There were no real differences in tenderness scores due to the method of stilbestrol administration.

Analysis of scores of the individual cuts revealed no significant differences in juiciness among the four groups (table 2). When the scores of four cuts were combined, the difference in juiciness scores between Groups III and IV was significant. The stilbestrol-treated group had slightly lower juiciness scores than Group III, but higher than Group IV. There were significant differences in juiciness between the round and rib roasts, but none between any two of the same types of roasts, although the posterior round tended to be less juicy than the anterior, and the 9–10–11th ribs slightly less juicy than the 6–7–8th. (table 4).

TABLE 4. AVERAGE SCORES OF FOUR CUTS

	Cuts				Statistics		
						L.S.D.	
Factor	Round (anterior)	Round (posterior)	Ribs 9–10–11	Ribs 6–7–8	"F" ratio	.05	.01
Tenderness	4.55	3.96	5.68	5.81	103.75***	0.37	0.42
Juiciness	4.16	3.91	4.96	5.18	64.34***	0.30	0.37
Flavor	4.55	4.40	5.22	5.27	50.71***	0.24	0.30

*** Significant at .001 probability level.

Significant differences in flavor scores among the four groups appeared only in the 9–10–11th rib cut. Group IV scored less flavorful than the other groups (table 2). Analysis of the combined scores of the four cuts resulted in significant differences in flavor between Group IV and each of the more highly finished groups. No differences in flavor were noted between the two stilbestrol-treated groups nor between either of these groups and Group III. Flavor quality among the four cuts varied significantly, with obvious differences between the top round and rib roasts (table 4). As with the tenderness and juiciness factors, the posterior round cut was scored lower than the anterior for the flavor factor.

For any of the factors evaluated, the effect of the treatment applied to each of the groups was the same in each cut. Because there was no interaction effect of treatment and cut, the quality scores for the four cuts of each animal were combined to determine the relationship between the objectively measured quality factors and the scores ascribed by the sensory panel. The correlation coefficients of these relationships are presented in table 5. The juiciness-moisture relationship, although statistically significant at the 5% probability level as determined by the "t" test, was not outstanding. However, the correlation coefficient of the flavor-ether extract

measurements was highly significant. This relationship has been found in other investigations (Cover *et al.*, 1956; Simone *et al.*, 1958).

Summary

Tenderness, juiciness and flavor factors were measured by a sensory panel on meat from four groups of Hereford steers; Group I, stilbestrol administration orally in daily feed, choice grade finish; Group II, stilbestrol administration by pellet implantation, choice grade finish; Group III, no hormone treatment, choice grade finish; Group IV, no hormone treatment, below choice grade finish. Results of evaluation of anterior and posterior top round roasts and 6–7–8th and 9–10–11th rib roasts indicated that hormone treatment by either method of administration produces beef which is less tender than beef from untreated steers which are finished to the same degree. Hormone treatment did not affect the juiciness and flavor qualities. Method of stilbestrol administration did not influence quality factors.

Both top round roasts were scored significantly lower in all factors than the rib roasts. The posterior rounds were less tender than the anterior, but no significant differences were found between the two rib roasts. Treatment effects were similar on all four cuts evaluated. A highly significant correlation was noted between the percent ether extract and flavor scores.

TABLE 5. CORRELATION COEFFICIENTS

Factor	Percent separable fat	Percent ether extract (marbling)	Percent moisture
Tenderness	0.127	0.155	0.268
Juiciness	.331	.334	—.353*
Flavor	.203	.769***	.119

* Significant at .05 probability level.
*** Significant at .001 probability level.

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STUDIES ON THE *IN VITRO* RUMEN PROCEDURE: IMPROVED INOCULUM PREPARATION AND THE EFFECTS OF VOLATILE FATTY ACIDS ON CELLULOSE DIGESTION [1, 2]

RONALD R. JOHNSON, BURK A. DEHORITY,[3] AND ORVILLE G. BENTLEY

Ohio Agricultural Experiment Station, Wooster [4]

IN recent years several investigators have used *in vitro* rumen fermentation techniques in an attempt to gain more knowledge of the complex processes involved in rumen digestion and metabolism. In the process of development of these *in vitro* studies certain methods have evolved which, in some cases, appear to account for the differences in results obtained by various investigators. Warner (1956) has classified the *in vitro* rumen procedures as follows: (1) Undiluted or only slightly diluted rumen liquor incubated with substrate in an all glass impermeable system (Pearson and Smith, 1943; Quin, 1943; Gray *et al.*, 1951); (2) whole rumen liquor diluted to half strength with mineral solution incubated with substrate in an impermeable system (Burroughs *et al.*, 1950a; McBee, 1953); (3) various fractions of rumen liquor used in impermeable systems, e.g., centrifuged cells and washed cell suspensions (McNaught, 1951; Cheng *et al.*, 1955; Bentley *et al.*, 1954a); and (4) rumen liquor, usually whole or undiluted, incubated with a substrate in a semipermeable container, dialyzing against a mineral solution (Louw *et al.*, 1949; Huhtanen and Gall, 1952; Wasserman *et al.*, 1952).

Since 1953, the method used in this laboratory has involved the use of rumen bacterial cells, separated from rumen liquor by centrifugation, as an inoculum for a medium consisting of a mineral mixture, certain organic growth factors, and carbohydrate substrates such as pure cellulose or starch (Bentley *et al.*, 1954a, 1955). Growth factors, non-protein nitrogen, fatty acids, in rumen liquor and various B-vitamins are variable. Since the rumen liquor is not part of the inoculum in this procedure, the effect of these variables, when present, is eliminated and this method therefore may be used to study the nutritive requirements and activities of the rumen bacteria in purified medium. This technique has also been used in the study of digestion of natural forages by rumen bacteria (Kamstra, 1955).

Improvements in the method of inoculum preparation and tests designed

[1] Approved for publication as Journal Article No. 29–58 by the Associate Director of the Ohio Agricultural Experiment Station. A preliminary report of this investigation was presented before the 49th meeting of the American Society of Animal Production, November, 1957, and the Abstract appears in the J. Animal Sci. 16:1088 (1957)

[2] The studies reported herein were supported in part by a Grant-in-Aid from Swift and Company, Chicago, Illinois.

[3] Present address: Department of Animal Industries, Connecticut Agricultural Experiment Station, Storrs, Connecticut.

[4] Department of Animal Science.

to evaluate this inoculum are reported in this paper. Also reported are experiments testing the effect of fatty acid end-products on the activity of rumen bacteria *in vitro*.

Experimental

Procedure. The general procedure used in these experiments was similar to that described previously by Bentley *et al.* (1955). The basal medium was the same as that of the latter investigators except that the cellulose [5] level was raised to 2 gm. per 100 ml. medium. Thirty mg. of valeric acid was added as a growth factor to the flasks which served as the positive controls. Cellulose digestion was used as the measure of bacterial activity and was determined by cellulose analysis according to the method of Hershberger *et al.* (1955).

Inoculum Preparation. The inoculum used previously (I) was prepared as described by Bentley *et al.* (1954a, 1955). Rumen liquor pressed from the rumen contents of a steer fitted with a permanent ruminal fistula was strained through cheese cloth and centrifuged in a Sharples super-centrifuge. The sediment was resuspended in phosphate buffer, pH 7.0, and 10 ml. (containing bacteria centrifuged from 40 ml. rumen liquor) was used to inoculate 100 ml. of basal medium.

In preparation of the improved inoculum (I_1) the liquor first expressed from the rumen contents was discarded and the remaining pressed pulp was mixed with phosphate buffer (the same as used in final inoculum preparation) in a ratio of 8 lb. of pressed pulp to 3 liters of buffer. This mixture was stirred moderately and resqueezed in the press. The liquor thus obtained was filtered through cheesecloth, centrifuged in the supercentrifuge and resuspended in the usual manner. A 10-ml. aliquot of this inoculum was equal to about 50 gm. of pressed pulp.

Washed Cell Study. Washed cell preparations were made by recentrifuging the final inoculum described above in the super centrifuge and then resuspending it in fresh buffer. Cells washed both one and two times were used as an inoculum for media with and without valeric acid.

Aeration and Incubation Studies. Samples of inoculum (I_1) were aerated by bubbling air through them at room temperature for varying periods of time up to 30 minutes and then were used to inoculate flasks containing valeric acid in the medium. Similar tests were performed using inoculum that had been pre-incubated at 37° C. for varying periods of time up to 6 hours.

Effects of Fatty Acid End Products. Acetic, propionic and butyric acids were added (as their sodium salts) to the medium both individually and in combination and both with and without added valeric acid.

[5] The cellulose used routinely in these experiments was Solka-Floc BW-40, obtained from Brown Company, Berlin, New Hampshire. Several other Solka-Floc samples were investigated but were not found to be as suitable for the volumetric cellulose determination.

TABLE 1. COMPARISON OF THE CELLULOLYTIC ACTIVITY OF FIRST EXTRACT (I) AND SECOND EXTRACT (I_1) INOCULA FOR THE *IN VITRO* RUMEN FERMENTATION

Inoculum	Cellulose digested, gm.[a]	
	No valeric acid[b]	Plus valeric acid[b]
I (First extraction)	0.02	0.72[c]
I_1 (Second extraction)	0.31[c]	1.20[c]
$I+I_1$	0.63[c]	1.26[c]

[a] Each value represents the average for three 30-hour experiments.
[b] Valeric acid is used as a growth factor for the cellulolytic rumen bacteria. Thus, the "no valeric" and "plus valeric" flasks comprise the negative and positive controls, respectively.
[c] $I+I_1$ (no v.a.) statistically different from I_1 (no v.a.) and I_1 (v.a.) statistically different from I (v.a.) both at the 1% level.
$I+I_1$ (v.a.) not statistically different from I_1 (v.a.).

Results

Inoculum Studies. The comparison of the cellulolytic activities of I and I_1 is given in table 1. Approximately 60% more cellulose was digested using I_1 than using I. Although the negative control (no valeric acid) for I_1 is higher than that for I, the difference between the negative and positive control (plus valeric acid) is greater for I_1. The combination of $I+I_1$ was no better than I_1 alone and gave a higher negative control.

When three levels of I_1 were tried (table 2) it was found that 10 ml. of inoculum (equivalent to 50 gm. pressed pulp) gave the best results in 30-hour experiments. At this level, cellulose digestion was near maximum and the negative control was the lowest.

Table 3 gives the results of the washed cell experiments. Washing the cells once reduced the cellulolytic activity to about one-third of the original and a second washing destroyed the cellulolytic activity entirely.

Figure 1 illustrates the effect of aeration of the inoculum, prior to inoculation, on the resulting cellulolytic activity in a 30-hour fermentation. Although the activity steadily decreased as aeration time increased, 60% of the activity still remained after 15 minutes of aeration. Similarly, pre-incubation at 37° C. (Figure 2) decreased the activity only 23% after 2

TABLE 2. *IN VITRO* CELLULOLYTIC ACTIVITY OF DIFFERENT LEVELS OF THE SECOND EXTRACTION INOCULUM (I_1)

Level of I_1[a]	Cellulose digested, gm. (30 hr.)	
	No valeric acid	Plus valeric acid
10 ml.	0.13	1.23
20 ml.	0.70	1.33
30 ml.	0.52	1.35

[a] 10 ml. equal to approximately 50 gm. pressed pulp.

TABLE 3. *IN VITRO* CELLULOLYTIC ACTIVITY OF WASHED CELL SUSPENSION PREPARED FROM SECOND EXTRACTION INOCULUM (I_1)

Inoculum[a]	Cellulose digested, gm. (30 hr.)	
	No valeric acid	Plus valeric acid
I	0.08	1.17
I_1 (washed once)	0.00	0.44
I_1 (washed twice)	0.00	0.00

[a] Previous experiments had shown similar results with washed suspensions of first extract inoculum (I).

hours. Further pre-incubation beyond 2 hours, however, decreased the activity rapidly.

The Effect of Fatty Acid End Products. Since no means of eliminating the end products of cellulose digestion is provided in this *in vitro* technique, it was of interest to test for inhibition by the major end products of carbohydrate digestion, i.e., the fatty acids. The results of these experiments are reported in table 4. When added individually, acetic and propionic acids were not inhibitory at levels of 300 mg. per 100 ml. of medium, nor was butyric at 30 mg. per 100 ml. As will be shown below, butyric acid actually possessed cellulolytic factor activity in the range from 50 to 150 mg./100

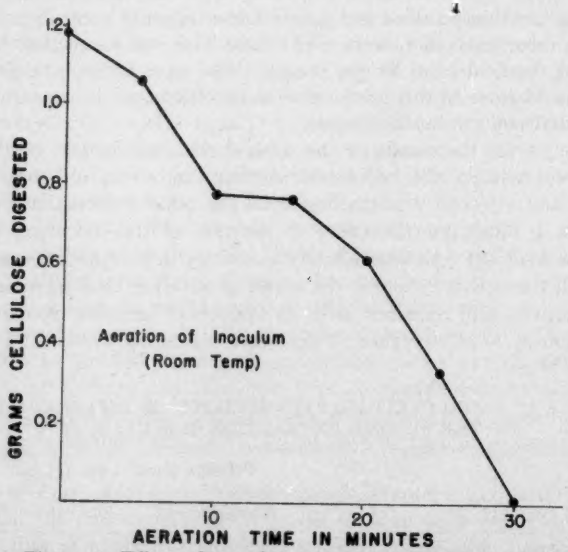

Figure 1. Effect of aeration at room temperature on the in vitro cellulolytic activity of the second extraction inoculum (I_1) when used in a 30-hour fermentation (average of three experiments).

ml. When acetic, propionic and butyric acids were tested as a mixture in a ratio of 10:10:1, respectively, the three acids were partially inhibitory at the 300:300:30 level and completely inhibitory at higher levels.

When the cellulolytic activity of short chain fatty acids was reported by this laboratory (Bentley *et al.*, 1954b, 1955), butyric acid was said to have very little cellulolytic factor activity in the range in which valeric acid was active (10 mg./100 ml. medium). Recent experiments, however, have shown that at higher levels, butyric acid promotes marked cellulolytic activity. The activities of C_2 to C_5 fatty acids are shown in Figure 3. These results show that the activity supported by 150 mg. of butyric acid per 100 ml. is nearly equal to that given by 30 mg. of valeric acid per 100 ml. Propionic acid sometimes exhibited slight cellulolytic activity but the effect was not marked, whereas acetic showed no cellulolytic activity. The mechanism by which valeric, butyric, and other fatty acids promote bacterial growth and cellulose digestion is unknown as yet.

Discussion

In the study and development of the *in vitro* rumen fermentation procedure used at the Ohio Station, continuous attempts have been made to

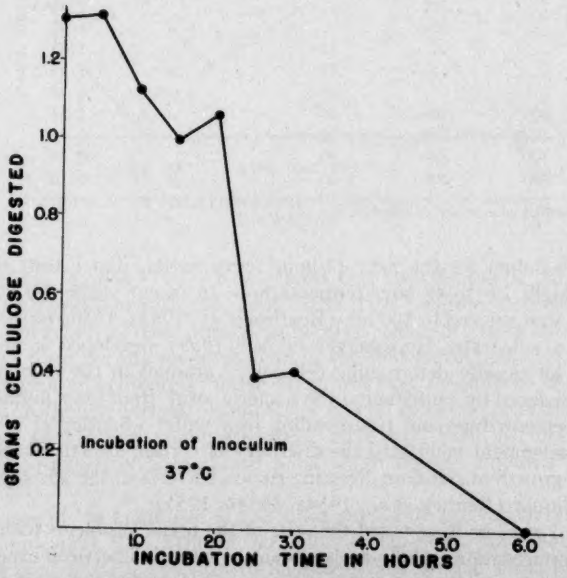

Figure 2. Effect of pre-incubation at 37°C. of the second extraction inoculum (I_3) on the in vitro cellulolytic activity in a 30-hour fermentation (average of three experiments).

improve the technique both in terms of accuracy and ease of manipulation. Originally, liter flasks containing suitable carbohydrate substrates plus a mineral mixture were inoculated with whole rumen liquor, and cellulose was determined by the Crampton and Maynard (1938) procedure (Burroughs *et al.*, 1950a, 1950b, 1950c; Arias *et al.* 1951). Attempts to eliminate inoculum variations were made by a process of four "serial transfers", in which half the broth from the preceding 24-hour fermentation was used

TABLE 4. THE INHIBITORY EFFECTS OF THE FATTY ACID END PRODUCTS ON CELLULOSE DIGESTION IN THE *IN VITRO* RUMEN FERMENTATION

Addition to medium[a] mg./100 ml.			
Acetic acid	Propionic acid	Butyric acid	Cellulose digested gm.
0	0	0	1.23
100	...	...	1.47
200	...	...	1.40
300	...	...	1.37
...	100	...	1.37
...	200	...	1.35
...	300	...	1.27
...	...	10	1.35
...	...	20	1.33
...	...	30	1.38
100	100	10	1.35
200	200	20	1.32
300	300	30	0.35
400	400	40	0.16

[a] Basal medium plus 30 mg. of valeric acid.

as the inoculum for the next 24-hour fermentation. Later tests were run with straight 24- to 48-hour fermentations. In recent years, the volume of medium was reduced to 100 ml. (Bentley *et al.*, 1955). Using purified cellulose as a substrate, Hershberger *et al.* (1955) developed a volumetric method for rapidly determining cellulose. Variables in the inoculum were further reduced by centrifuging the bacteria away from their mother liquor in a supercentrifuge and resuspending in a buffer (Bentley *et al.*, 1954). This development facilitated the discovery of certain growth factors necessary for growth of cellulose digesting rumen bacteria in the absence of their mother liquor (Bentley *et al.*, 1954a, 1954b, 1955).

Several workers have noted that one of the major problems with *in vitro* rumen fermentation techniques has been the variation between experiments. Since most investigators are now using purified media and purified carbohydrate substrates, such as cellulose or starch, the main contributor to this variability is the inoculum, or its source. Attempts to minimize this

variability have been made by using the same animal in all experiments, maintaining the animal on a uniform ration, withholding the feed and water for certain periods of time prior to sampling, as well as by use of the methods of inoculum preparation described in the introduction. Although these precautions were quite successful in reducing the variation, the variation was still not completely eliminated. It was noted, during the course of the studies reported herein, that not only did I_1 give better cellulose digestion but exhibited much less variation between experiments than previous inoculum preparations.

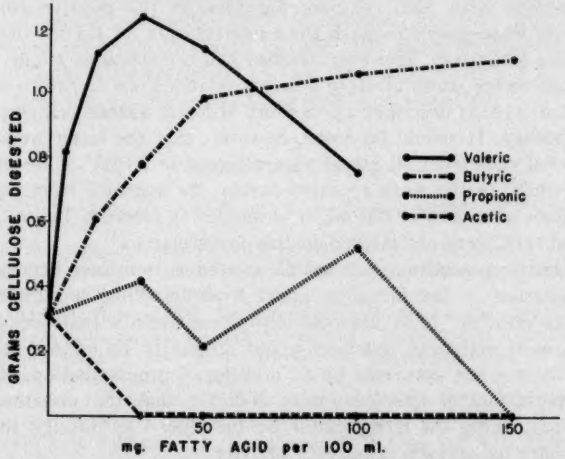

Figure 3. In vitro cellulolytic factor activity of C_2—C_5 fatty acids.

Baker and Harriss (1947) have shown that the cellulolytic bacteria are intimately associated with the plant fibers. It is possible then that in the second extraction of the pressed pulp (I_1), more of these bacteria are separated from the fibrous mass than in the first extraction (I). A better representation of cellulolytic active organisms may possibly be obtained in this way. Although adequate numbers of crucial tests on the ability of this inoculum to support starch digestion have not been performed, this digestive process has been shown to occur in normal amounts in a few experiments.

Since one of the main uses of the inoculum was in growth factor studies, it was important that little or no cellulose digestion take place in the negative control flask (no growth factor added), or that large differences exist between the positive and negative controls. These conditions afford a more critical test on substances possessing low factor activity. Thus it

is important that the inoculum be kept as free as possible from plant or feed debri or excess bacterial cells that might contain growth stimulating amino acids or other stimulatory factors and yet contain enough live bacterial cells to establish an optimum growing culture. Many similar observations have been made in microbiological assay procedures using pure cultures. The 10 ml. level of the inoculum I_1 approaches these conditions.

It would appear that washed cell preparations are not a suitable inoculum for the *in vitro* rumen procedure described herein. Since, in using inoculum I_1, low cellulose digestion was observed in the negative control in conjunction with high cellulose digestion in the positive control, it would seem unnecessary to wash the bacterial cells for the studies underway in this laboratory. However, washed cell preparations might be very appropriate in the study of other nutrient relationships in rumen bacteria. Cheng *et al.* (1955) describes a procedure utilizing washed cell suspensions as an inoculum. It should be noted, however, that the latter worker used the bacterial cells from 40 ml. of rumen liquor in 20 ml. of fermentation medium while, in the work reported herein, the bacteria from 40 ml. of rumen liquor were used in 100 ml. of fermentation medium. Larger aliquots of washed cells were not studied in this investigation.

No elaborate precautions against air contamination have been taken in the preparation of the inoculum apart from handling as carefully and quickly as possible. It is obvious from these results that for cellulose digestion more elaborate practices would appear to be unnecessary since the activity was not decreased by 30 minutes of pre-incubation. However, other experiments in this laboratory indicate that the maintenance of anaerobiosis during the fermentation by bubbling Co_2 through the flasks was necessary for uniform cellulolytic activity.

As shown in table 4 the presence of high levels of fatty acid end-products in the flasks can inhibit cellulose digestion. However, it should be noted that the levels found to be inhibitory, i.e., 300:300:30, are above those levels usually produced during a 30-hour *in vitro* fermentation using cellulose as a substrate in our laboratory. For example, levels of 50 to 200 mg. of acetic acid, 100 to 200 mg. of propionic acid, and 10 to 50 mg. of butyric acid per 100 ml. are commonly found in such *in vitro* experiments (Hershberger *et al.*, 1956). In a longer fermentation, the levels may conceivably reach an inhibitory range. Indeed, levels considerably greater than these are found in natural rumen liquor. Annison (1954) reports acetic acid levels ranging from 250 to 600 mg./100 ml., propionic acid from 118 to 300 mg./100 ml. and butyric from 30 to 250 mg./100 ml. in rumen liquor from sheep on different diets. Balch and Rowland (1957) report similar values for rumen liquor in dairy cattle. Several other workers have reported levels falling within these ranges.

Summary

An improved inoculum for *in vitro* rumen fermentations has been prepared by discarding the first extraction of the rumen contents, resuspending the pressed pulp in buffer, and re-expressing this liquor as an inoculum source. Inoculum prepared from this liquor gave higher cellulose digestion and less variation between experiments. The activity of these cells was destroyed completely by washing twice with buffer. Aeration for 15 minutes destroyed only 40% of the activity of this inoculum while incubation at 37° C. for 2 hours destroyed only 23% of the activity.

A mixture of acetic, propionic and butyric acids (300, 300, and 30 mg./per 100 ml., respectively) inhibited cellulose digestion. Butyric acid alone, however, at levels of 100 to 150 mg./100 ml. of medium possessed cellulolytic-factor activity similar to that demonstrated by 10 to 30 mg. of valeric acid. The significance of these findings is discussed.

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PERFORMANCE OF STILBESTROL TREATED STEERS AND LAMBS AS INFLUENCED BY TAPAZOLE[1]

J. W. Blair,[2] E. S. Erwin and C. B. Roubicek[3]

University of Arizona, Tucson[4]

In the past decade much interest has been directed toward increased production of meat animals through chemical stimulation. Inconsistencies have been reported regarding the value of antibiotics in ruminant rations (Behman *et al.*, 1955; Kunkel *et al.*, 1956; Johnson *et al.*, 1956). The variable results have been attributed among other things to environmental factors such as disease and nutritional stress.

Although it has been established that estrogenic compounds stimulate growth in ruminants, the lack of desirable finish in the carcass has been reported by Clegg and Cole (1954), Bell *et al.* (1954) and Cahill *et al.* (1954). Apparently, the growth stimulation by estrogen is primarily that of increased protein anabolism at the expense of fat.

Suppression of the thyroid or metabolic rate should cause a larger proportion of the net ingested calories to be anabolized into fat. Several years ago thiouracil, a goitrogen, was investigated for ruminants as a feed additive by Beeson *et al.* (1946). Inconsistent results with ruminants have been attributed to the unpalatability of the drug (Blaxter *et al.*, 1949).

1-Methyl, 2-mercaptoimidazole (commonly termed Tapazole) is probably the most potent and fastest acting antithyroid compound known. In these studies, Tapazole was fed to determine the modifying effect on stilbestrol treated lambs and steers. Because of the possible lowered resistance of the Tapazole-treated ruminants to environmental stress, the value of chlortetracycline was investigated.

Procedure

Trial I. During the winter of 1956, 118 cross-bred feeder lambs were numbered and randomly allotted to six pens of 15 and two pens of 14 animals. The statistical design was a 2 x 2 x 3 factorial, in a split-plot randomized block, with two replications of the following dietary treatments: (1) Control, (2) Tapazole, (3) chlortetracycline and (4) Tapazole and chlortetracycline. The lambs in each pen were randomly divided into three equal sub-treatment groups and subcutaneously implanted at the base

[1] Diethylstilbestrol pellets used in this experiment were furnished by Chas. Pfizer & Co., through the courtesy of W. C. Sherman; the 1-Methyl, 2-Mercaptoimidazole was furnished by Eli Lilly & Co., through the courtesy of C. F. Chappel and T. M. Means; and, Chlortetracycline was furnished by the American Cyanamid Company.

[2] Conducted in partial fulfillment for the degree of Master of Science in Animal Science.

[3] The authors acknowledge the statistical assistance of H. Tucker, Experiment Station Statistician.

[4] Department of Animal Science, Arizona Agricultural Experiment Station Technical Paper No. 457.

of the ear with the following amounts of stilbestrol: None, 3 mg. and 6 mg. The dietary treatments were randomly assigned by pens and the lambs were allowed 5 days adjustment to the ration. At this time the lambs were individually weighed and implanted with stilbestrol.

The all-pelleted ration, shown in table 1, was fed *ad libitum* to all lambs. For the respective dietary treatments, chlortetracycline and Tapazole were incorporated at the rates of 7.0 mg. and 3.75 mg. per pound of feed, respectively.

TABLE 1. CONSTITUENTS OF THE EXPERIMENTAL BASAL RATION IN TRIALS I AND II

Feed constitutents[a]	%	Chemical constituents	%
Cotton gin trash	33.2	Moisture	5.70
Cane molasses	8.3	Protein	13.90
Barley	19.5	Crude fiber	14.15
Milo	19.5	Ether extract	1.43
Cottonseed meal	10.5	N.F.E.	55.09
Dehydrated alfalfa meal	8.0	Ash	9.73
Trace mineral salt	0.5	Lignin	7.85
Bone meal	0.5		
Anise (8 gm./ton)			

[a] Compounded into one-half inch pellets.

Twenty-four hour fecal collections were taken over a period of 6 days, beginning on the 30th day of the experiment from six lambs of each of the sub-treatment groups that were implanted with none and 6 mg. of stilbestrol. All rations and feces were analyzed for moisture, protein, crude fiber, ether extract, ash, nitrogen-free extract and lignin by approved A.O.A.C. procedures (table 1). Apparent digestibility coefficients were determined by means of the lignin ratio technique as outlined by Schneider *et al.* (1955).

At 54 days, five of the heaviest lambs were selected for slaughter from each sub-treatment group, two from one replication and three from the second (Group A). The remaining 58 animals, designated as Group B, were individually weighed and marketed at 75 days. All results, including feed utilization, weight gains, carcass grade and dressing percent, were subjected to analyses of variance using unweighted sub-treatment means (Snedecor, 1955).

Trial II. During the late summer of 1957, 40 ewe and 40 wether lambs were randomly allotted to eight pens of five ewe and five wether lambs each. All lambs were subcutaneously implanted at the base of the ear with 3 mg. of stilbestrol. The statistical design was a 2 x 4 factorial, in a split-plot randomized block that incorporated two replications of the following dietary Tapazole treatments imposed on both sexes: None, 3.5 mg., 7.0 mg. and 14.0 mg. per pound of feed. All rations were formulated to contain

7.0 mg. of chlortetracycline per pound. The composition of the basal ration fed *ad libitum* for 68 days in this trial was identical with the ration fed in Trial I (table 1). The values for total gain, feed utilization, carcass grade and dressing percent were subjected to analyses of variance (Snedecor, 1955).

Trial III. During the early fall of 1957, 72 yearling Hereford steers were randomly allotted to six pens of 12 animals each and fed a ration calculated on a dry-weight basis of 50% concentrate pellets, 40% chopped alfalfa hay and 10% hegari silage for 93 days. The pelleted concentrates contained 45% barley, 50% milo, 4% cottonseed meal, 1% salt and chlortetracycline (8 mg./lb.). The steers were fed twice daily in accord with consumption.

The statistical design was a split-plot randomized block that incorporated a factorial of two replications of three levels of dietary Tapazole and three levels of implanted stilbestrol. Tapazole was premixed with cottonseed meal and salt and incorporated into the concentrate pellets in the following amounts per pound: None, 14 mg. and 28 mg. The steers in each pen were randomly divided into three equal sub-groups and implanted at the base of the ear with the following amounts of stilbestrol: 6 mg., 18 mg. and 30 mg.

The values of total gain, carcass grade and dressing per cent were subjected to analyses of variance (Snedecor, 1955).

Results and Discussion

Trial I. The data on the performance of the lambs in Trial I and the appropriate statistical analyses are summarized in tables 2 and 4, respectively. When all levels of implanted stilbestrol were considered neither chlortetracycline nor Tapazole fed singly or in combination to lambs significantly influenced rates of gain. Jordan and Bell (1954) and Kunkel *et al.* (1956) obtained no effect from feeding antibiotics and Barrick *et al.* (1949) reported no differences between weight gains when various amounts of the goitrogen, thiouracil, were fed to lambs.

In this study, the average rates of gain of the lambs implanted with 3 mg. of stilbestrol was 12% higher than that of the lambs implanted with 6 mg. This was due to an interaction effect of the 3 mg. level of implanted stilbestrol with dietary Tapazole (table 4). This interaction will be discussed later. When Tapazole was not included in the ration, the growth stimulations due to the implantation of 3 mg. and 6 mg. of stilbestrol were similar.

No significant differences were found between the rates of gain of implanted lambs fed with and without chlortetracycline for 54 days (Group A). However, table 4 shows a significant antibiotic-stilbestrol interaction in the growth rate of lambs fed for 75 days (Group B); chlortetracycline-fed lambs implanted with 6 mg. of stilbestrol gained at a faster rate than

TABLE 2. INFLUENCE OF CHLORTETRACYCLINE, TAPAZOLE AND STILBESTROL IMPLANTATION ON FATTENING LAMBS[a]

Implanted stilbestrol, mg.	No. lambs	Average initial wt., lb.	Average daily gain, lb.	Feed/ lamb/ day, lb.	Feed/lb. gain, lb.	Average carcass grade[b]	Average dressing %[c]
			Control				
0	10	67.0	.534			2.2	50.2
3	9	69.6	.563			2.0	49.2
6	10	68.8	.578			2.4	50.8
Average		68.4	.558	3.85	6.90	2.2	50.1
			Chlortetracycline				
0	10	62.8	.454			2.4	52.6
3	10	64.6	.577			2.2	50.5
6	10	65.0	.611			2.7	48.3
Average		64.1	.528	3.69	6.89	2.4	50.5
			Tapazole				
0	10	64.8	.445			2.1	50.9
3	10	61.7	.600			2.1	48.7
6	9	61.4	.512			2.8	49.2
Average		62.7	.519	3.87	7.44	2.3	49.0
			Chlortetracycline+Tapazole				
0	9	60.1	.412			2.4	50.5
3	10	67.8	.701			2.3	48.2
6	10	68.7	.478			2.2	50.1
Average		65.7	.576	4.02	7.47	2.3	49.6

[a] Combined results of all lambs.
[b] The numbers 1, 2, 3 and 4 were assigned to high choice, low choice, high good and low good, respectively.
[c] Calculated from off-truck weights and chilled-carcass weights.

those implanted with 3 mg. The lack of an antibiotic response in lambs of Group A may have been due, in part, to the fact that those animals were thriftier and less susceptible to subclinical disease. The relatively large error (table 4) of Group A as compared to Group B is indicative of more homogenous growth of the lambs that were fed for 75 days. The lack of statistically significant antibiotic response may have been due to the large individual variation in growth rate of lambs in Group A. Jordan *et al.* (1956) observed no differences in lamb performance when stilbestrol and chlortetracycline were fed singly or in combination.

Dietary Tapazole significantly altered the weight gains of stilbestrol-implanted lambs (tables 2 and 4). Tapazole-fed lambs implanted with 3 mg. stilbestrol gained faster than animals in any other group treated with stilbestrol and Tapazole combinations. In contrast, Tapazole-fed lambs implanted with 6 mg. of stilbestrol gained only slightly more than the non-implanted Tapazole-fed lambs; neither group gained as fast as the group that received no Tapazole or stilbestrol. Apparently, the value of dietary Tapazole for increased lamb gains was dependent upon the level

of implanted stilbestrol. Although the lambs treated with 3 mg. of stilbestrol and fed the combination of chlortetracycline and Tapazole gained faster than any other treated group, no interaction of chlortetracycline and Tapazole was revealed in the statistical analysis (table 4). These data indicate that the variation in the magnitude of stilbestrol response reported by Clegg and Cole (1954) and Andrews *et al.* (1956) may be due, for the most part, to the variable metabolic rates of the experimental animals.

Since stilbestrol-implanted and non-implanted lambs were fed together by pen, only the influence of dietary additives on feed consumption could be measured (table 2). No significant difference was found (table 4). Feed consumption was increased slightly by the addition of Tapazole; therefore, Tapazole at the level used in this study did not possess the unpalatability that Andrews *et al.* (1947) reported for thiouracil.

The influence of Tapazole, stilbestrol and chlortetracycline, singly and in combination, on nutrient digestion was determined simultaneously with the growth study to determine whether the growth alteration could be attributed, in part, to digestibility (table 3). The greatest mean difference between treatments occurred in crude fiber digestion; this difference did not approach statistical significance. It was concluded that treatment did not alter nutrient digestion and that the growth alteration was due only to metabolic changes.

TABLE 3. THE INFLUENCE OF CHLORTETRACYCLINE, TAPAZOLE AND STILBESTROL IMPLANTATION ON DIGESTIBILITY OF NUTRIENTS BY FATTENING LAMBS (TRIAL I)

Implanted stilbestrol, mg.	No. lambs	Digestion coefficients					
		Dry matter	Protein	Crude fiber	Ether extract	Ash	Nitrogen-free extract
				Control			
0	4	62.74	56.38	28.19	75.82	47.58	76.01
6	5	63.64	54.03	27.17	77.82	53.05	77.39
Average		63.24	55.07	27.62	76.93	50.62	76.77
				Chlortetracycline			
0	6	62.10	55.45	24.50	71.97	62.56	76.23
6	6	63.21	57.40	27.87	73.09	54.99	75.57
Average		62.66	56.42	26.18	72.53	53.77	75.90
				Tapazole			
0	6	62.42	55.88	23.90	77.29	53.34	75.74
6	6	63.60	56.39	23.31	75.44	53.75	77.23
Average		63.01	56.13	23.60	76.36	53.54	76.48
				Chlortetracycline+Tapazole			
0	5	63.37	56.39	23.31	75.44	53.75	77.23
6	6	62.44	55.86	22.66	63.88	55.20	76.01
Average		62.91	56.10	22.95	69.13	54.54	76.56

Carcass characteristics were not influenced by any treatment (tables 2 and 4). Jordan (1953) found that 6 mg. of stilbestrol implanted in lambs lowered carcass grade. Furthermore, the other commonly reported side effects from stilbestrol were not observed in any lamb in this study.

TABLE 4. ANALYSES OF VARIANCE (TRIAL I)

Source[a]	d.f.	Mean squares				
		Total gain all lambs (54 days)	Total gain group B (75 days)	Feed/lb. gain, lb.	Carcass grade	Dressing %
Replicates	1	12.54	1.13	0.35	0.0008	4.34
Diets	3	27.11	26.44	0.43	0.0561	1.83
C	(1)	35.57	23.06			0.80
T	(1)	0.58	48.94			4.68
CT	(1)	44.61	5.07			0.01
Error (A)	3	45.20	94.50		0.8381	1.86
DES	2	155.42**	212.63**		0.3056	8.77
L	(1)	211.41**	145.69**		0.2258	10.08
Q	(1)	99.73*	280.13**		0.3852	6.52
DEX x Diets	6	25.37	60.75**		0.1075	2.98
CL	(1)	23.32	91.69**			
TL	(1)	0.87	0.56			
CTL	(1)	0.87	7.88			
CQ	(1)	2.04	52.31**			
TQ	(1)	111.68*	209.81**			
CTQ	(1)	13.41	0.06			
Error (B)	8	11.08	1.86		0.1837	4.34
Within animal variation	94	69.45		0.20[b]	0.0986	1.18
	34		8.68			

* $P<.05$.
** $P<.01$.
[a] C=Chlortetracycline; T=Tapazole; DES=Stilbestrol; L=DES Linear; Q=DES Quadratic.
[b] Three degrees of freedom.

Trial II. This trial was designed to study the interaction of Tapazole and stilbestrol on both ewe and wether lambs. The level of dietary Tapazole did not significantly alter the growth rate of lambs. The stilbestrol-treated ewe lambs that received 7.0 mg. of Tapazole per pound of feed gained less than ewe lambs fed either the higher or lower goitrogen level (table 6). This difference could be attributed only to chance variation. Although not statistically significant, there was a definite trend for both sexes implanted with stilbestrol and fed 3.5 mg. of Tapazole per pound of feed to gain faster than the control lambs. Henneman *et al.* (1955) reported that thyroxine production by animals varied with the season of the year. Since Trial I was conducted in mid-winter and Trial II in the late summer, the difference in the magnitude of Tapazole response in stilbestrol-implanted lambs could be attributed to season or environmental temperature.

TABLE 5. INFLUENCE OF VARIOUS LEVELS OF TAPAZOLE ON STILBESTROL IMPLANTED EWE AND WETHER LAMBS

Items	Tapazole per lb. ration, mg.			
	0	3.5	7.0	14.0
Number of lambs				
Ewe	10	10	10	10
Wether	10	10	10	10
Average initial weight				
Ewe	64.8	62.0	64.6	64.7
Wether	61.3	57.6	58.8	60.1
Average daily gain, lb.				
Ewe	0.557	0.573	0.579	0.552
Wether	0.586	0.607	0.494	0.547
Feed per lamb daily, lb.	4.35	4.20	4.14	3.92
Feed per lb. gain, lb.	7.60	7.12	7.71	7.12
Average carcass grade [a]				
Ewe	2.0	2.2	2.5	2.6
Wether	2.2	2.5	2.1	2.7
Average dressing percent [b]				
Ewe	55.05	54.46	53.93	55.54
Wether	55.07	55.39	54.31	54.31

[a] The numbers 1, 2, 3, 4 and 5 were assigned to the grades prime, high choice, average choice, low choice and good, respectively.
[b] Calculated from off-truck line weights and warm-carcass weights.

Although the lambs in Trial I consumed slightly more feed when Tapazole was incorporated in the ration, in Trial II there was a progressive decrease in feed consumption as the amount of Tapazole was increased in the ration (table 5). These data add to the possibility that the season or environmental temperature may alter the response of animals to goitrogenic materials. As in Trial I, Tapazole did not significantly alter the dressing percentage or federal carcass grades of the lambs.

TABLE 6. ANALYSES OF VARIANCE, TRIAL II

Source	d.f.	Mean squares			
		Total gain, lb.	Lb. feed/ lb. gain	Carcass grade	Dressing %
Replicates	1	19.20	0.432	0.20	2.10
Tapazole	3	51.66	0.201	1.03	3.56
Error (A)	3	32.60		1.50	1.31
Sex	1	4.50		0.45	0.00
Sex x Tapazole	3	70.76		0.35	4.5
Error (B)	4	53.67		0.83	1.19
Within animal variation	64	52.88		0.48	4.22

* $P<.05$.
** $P<.01$.

TABLE 7. THE INFLUENCE OF TAPAZOLE AND STILBESTROL ON FEEDLOT STEER PERFORMANCE

Tapazole/lb. concentrate, mg.	0	0	0	14	14	14	28	28	28
Stilbestrol, mg	6	18	30	6	18	30	6	18	30
No. of steers	8	8	8	8	7	8	8	8	8
Av. initial wt., lb.	727.5	680.6	695.6	718.1	739.0	738.1	685.6	706.2	698.7
Av. daily gain, lb.	2.31	2.93	2.94	2.43	3.16	2.94	2.35	2.82	2.80
Daily feed consumption/ steer, lb.									
Hegari silage		1.94			1.95			1.91	
Alfalfa hay		8.10			8.16			7.99	
Concentrates		10.31			10.60			10.16	
Total		20.35			20.71			20.06	
Feed/lb. gain, lb.[a]		7.40			7.37			7.56	
Transit shrinkage, %	6.98	6.69	7.28	6.95	6.07	9.02	6.43	6.46	6.71
Carcass data									
Grade[b]	3.88	2.75	2.38	2.75	2.00	2.00	3.12	2.75	2.38
Dressing percent[c]	62.1	62.9	62.5	62.0	62.4	63.5	61.8	61.9	62.3

[a] Based on dry matter, calculated as follows: Silage, 30%; hay, 95%; concentrates, 95%.
[b] The numbers 1, 2, 3, 4 and 5 were assigned to the grades high choice, average choice, low choice, high good and average good, respectively.
[c] Calculated from off-truck live weights and warm-carcass weights.

Trial III. The steers implanted with 18 and 30 mg. of stilbestrol gained significantly faster than those animals treated with 6 mg. (tables 7 and 8). When averaged over all dietary treatments, the implantation of 18 and 30 mg. of stilbestrol resulted in comparable stimulation of weight gains. Similar results were found with the lambs implanted with 3 and 6 mg. of stilbestrol in Trial I. The similar rates of gain in the 18 and 30 mg. stilbestrol-treated steers could be attributed to the relatively short duration of the trial (93 days). Perry *et al.* (1958) found that 12 and 24 mg. of stilbestrol stimulated steer gains similarly for 60 to 90 days while 36 mg. was most satisfactory for longer feeding periods.

Neither level of Tapazole fed to steers in this trial significantly altered

TABLE 8. ANALYSES OF VARIANCE (TRIAL III)

		Mean squares		
Source	d.f.	Total gain, lb.	Dressing %	Carcass grade[b]
Replicate	1	32.0	17.80	0.50
Tapazole	2	1608.5	3.08	3.50
Error (A)	2	129.5	0.79	2.00
Stilbestrol				
Linear	(1)	26602.0**	8.00	12.00**
Quadratic	(1)	13689.0**		1.00
Stilbestrol x Tapazole	4	287.8	1.39	0.50
Error (B)	6	536.2	4.84	1.50
Within animal variation	54	1155.0	3.42	1.42

* $P<.05$.
** $P<.01$.

the rate of gain (tables 7 and 8); however, the animals that were fed the lower level (148 mg. per steer daily) gained more than the control steers. Although the interaction effect of Tapazole and stilbestrol did not approach significance (table 8) the fastest rates of gain were made by the steers implanted with 18 mg. of stilbestrol and fed the low level of Tapazole. Significantly faster gains resulting from feeding Tapazole to stilbestrol implanted animals were obtained only in Trial I but the same trend was apparent in Trials II and III. These data indicate that the value of Tapazole may not only be limited to the level of administration but also to the amount of stilbestrol implanted in ruminants.

No apparent difference between feed consumption or efficiency of feed utilization could be attributed to Tapazole feeding. Consequently, the amounts of Tapazole fed to lambs or steers in these trials did not present the palatability problem reported for thiouracil by Blaxter *et al.* (1949).

Carcass grades were significantly increased with increasing amounts of stilbestrol (table 8). These data were not in agreement with the majority of other results; however, the stilbestrol treatment increased the carcass grades regardless of the dietary treatment (table 7). Although Tapazole feeding did not significantly alter weight gains, the carcasses of steers fed the low level (148 mg. per steer daily) were graded three-fourths of a grade higher than the control carcasses. The steers fed the high Tapazole level (284 mg. per steer daily) graded approximately one-half of a grade higher than those of the control animals (table 7). Andrews *et al.* (1947) reported that thiouracil-fed lambs exhibited larger amounts of internal and cod fat than the control animals. Whereas Beeson *et al.* (1946) obtained increased weight gains in thiouracil-fed steers, carcass condition was not affected. Shultze *et al.* (1950) reported no difference in growth or amount of carcass fat in thiouracil-fed dairy calves.

The beneficial effects from feeding goitrogenic materials become dependent upon the level of administration. Studies by Van der Noot *et al.* (1948) indicated that severe thyroidal depression resulted in decreasing normal rates of gain. The levels of Tapazole fed to the respective groups of steers in this trial were estimated from the amounts that were administered to the lambs during Trials I and II. The performance of ruminants to a fixed goitrogen intake may be dependent upon their initial thyroidal activity and nutritional and environmental factors that affect their general metabolic rate. The lamb response in Trials I and II exemplifies the inconsistency in performance of animals to Tapazole. The many factors that may influence the response of ruminants to Tapazole merit investigation.

Summary

A goitrogen, 1-methyl, 2-mercaptoimidazole (commonly termed Tapazole), was fed to 118 lambs in Trial I, 80 lambs in Trial II and 72 steers in Trial III to determine possible modifying effects on the performance of

ruminants implanted with stilbestrol. Also, the value of chlortetracycline fed in combination with Tapazole to stilbestrol-implanted lambs was investigated.

In Trial I the amount of stilbestrol implanted governed the response of lambs to dietary Tapazole; whereas 3 mg. of stilbestrol markedly increased gains, 6 mg. did not. Similar relationships of stilbestrol and Tapazole were found in Trials II and III.

A digestibility study was conducted in Trial I. Neither Tapazole, stilbestrol implantation nor chlortetracycline altered digestibility of the ration.

Carcass characteristics of stilbestrol-treated lambs fed Tapazole were unaffected. Tapazole administration to stilbestrol-implanted steers did not substantially increase rates of gain; however, carcass grade was markedly improved. Feed consumption and efficiency of utilization by the animals in three trials were not altered by the addition of Tapazole to the rations. No evidence of unpalatability was observed. The stimulation of animal performance by Tapazole varied greatly between trials; environmental differences that altered metabolic rate may have been responsible for the variation.

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THE RATE AND ECONOMY OF PIG GAINS AND THE REPRODUCTIVE BEHAVIOR IN SOWS WHEN LITTERS ARE WEANED AT 10 DAYS, 21 DAYS, OR 56 DAYS OF AGE [1]

H. L. SELF AND R. H. GRUMMER

University of Wisconsin, Madison [2]

INCREASING litter size or shortening the interval between litters should result in greater yearly production per sow unit. Observations by swine producers and research workers (Burger, 1952) indicate that most sows do not exhibit a fertile heat during lactation; however, sows may exhibit a sterile heat within 5 days following parturition (Warnick *et al.*, 1950; Baker *et al.*, 1953). Removing the pigs earlier than the customary 56 days of age to allow the sow to be rebred sooner necessitates special care of the pigs, depending on the age at which they are removed. Weybrew *et al.* (1949) reared pigs in cages on liquid milk-substitute diets from 2 days of age. Sheffy *et al.* (1951, 1952) reared pigs from birth and from 2 days of age on liquid rations high in fat content and found cleanliness difficult to maintain. Grummer (1954) reported satisfactory gains on dry rations when pigs were weaned at any time from one week to 8 weeks of age, but his studies suggested the earliest practical time to wean was 3 weeks of age. In subsequent trials (Grummer—unpublished) 3-week-old crossbred pigs of uniform size (8 to 10 pigs per pen) consistently gained at the rate of approximately 0.75 lb. per pig daily and averaged 1.0 lb. of gain per 2.25 lb. of feed from 3 weeks to 8 weeks of age. (Rations-table 1). The pigs were kept inside on concrete. Heat lamps were provided and the room temperature was maintained at 65° F. or above by a central heating system.

Factors other than growth that require consideration in practicing early weaning are total feed requirements (both the sow and litter), weight loss in sows, seasonal trends in the market for sows, the reproductive behavior of the sows, and the facilities available.

This study was undertaken to observe the results when relatively inexpensive rations were fed to early-weaned pigs kept under ordinary field conditions and to observe sow reproductive behavior when the litter was removed at 10, 21, or 56 days of age.

Materials and Methods

In order to observe, under field conditions, the economic factors involved in 10-day and 3-week weaning as compared to 8-week weaning and to

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[2] Department of Animal Husbandry.

obtain information on the reproductive behavior of sows when their pigs were removed at these ages, the studies were conducted at the University purebred herd facilities. Thirty-five sows (21 Poland China and 14 Chester White) from the University herd, ranging in age from 1 to 4 years, were used. They farrowed purebred litters between April 29 and May 30, 1955. Ten sows and litters were used in obtaining data when the litters were removed at 10 days of age. The remaining 25 litters were used for the growth and economic studies. Twenty-four of the sows were used for

TABLE 1. COMPOSITION OF RATIONS

Ingredient	Type of ration	
	Pig starter	Pig creep
	(lb.)	(lb.)
Corn	142.5	300.0
Soybean meal	140.0	100.0
Oat groats (rolled)	.....	135.0
Dried skimmilk	100.0	
Fish meal	12.5	
Dried whey	12.5	
Lard	25.0	
Dicalcium-phosphate	6.0	
Calcium carbonate	2.5	
Trace mineralized salt	3.0	3.0
Antibiotic (chlortetracycline) [a]	10.0	...
Vitamin premix	2.5	25.0 [b]
Sugar (sucrose)	50.0	...
Bone meal	....	3.0
Gr. limestone	....	2.0
Total	501.5	568.0

[a] 3.6 gm. per lb.
[b] Protein carrier containing 2 gm. of chlortetracycline per lb. and sufficient quantities of vitamins to meet the levels recommended by the National Research Council (1953).

reproductive behavior studies at the two later weaning stages. One sow in the 56-day group was removed from the reproductive studies at the time the litter was weaned. The first 13 sows to farrow were assigned to suckle their litters for 56 days; the other 12 were to suckle their litters for 21 days. A creep ration (see table 1) was available to all litters from approximately 10 days of age until 21 days of age. The 21-day weaned litters received a pig starter from the 21st to the 35th day and were then shifted to a creep ration from the 36th to the 56th day (table 1). The 56-day weaned litters were provided with a creep ration from approximately the 10th to the 56th day.

Sows were checked daily with a boar during the nursing and the post-lactation periods for evidence of estrus. Three sows (one Chester White and two Poland China) exhibited a post-partum heat; one each on the

2nd, 3rd and 5th day following farrowing. Previous reports, (Warnick *et al.*, 1950; and Baker *et al.*, 1953) indicate the post-partum heats to be sterile, therefore none of the sows were bred at these heats. Sows were mated once at the first post-weaning heat between approximately the 12th and 36th hour of heat. All sows in the 10-day weaned group and five sows in each of the other two groups were slaughtered 24 to 48 hours after the end of heat to obtain ovulation and fertilization data. Sows not slaughtered were rebred for fall farrowing.

The boars used in the fertility studies were purebreds of the same breed as the sow.

TABLE 2. REPRODUCTIVE PHENOMENA IN SOWS WHEN LITTERS ARE WEANED AT 10, 21, OR 56 DAYS OF AGE

Age of litter at weaning, days	10	21	56
Time from weaning to first post-weaning heat, days			
Mean	9.4*(10)[a]	6.2(12)	4.0(12)
Range	5 to 30	3 to 15	2 to 10
Mode	7	none	3
Average ovulation rate	12.8(8)[b]	15.2(5)	16.6(5)
Average fertilization rate[c]	93.4(8)	90.6(4)[d]	98.1(4)[e]

[a] No. in parenthesis indicates number of observations.
[b] Two of the original 10 sows were omitted due to completely cystic ovaries at autopsy.
[c] Fertilization rate $= \frac{\text{No. cleaved ova recovered}}{\text{No. ova recovered}} \times 100$.
[d] One sow omitted due to bilateral pyometra.
[e] One sow omitted due to failure to recover ova.
* The three groups differed significantly ($P<0.05$).

The litters of the 21-day weaned group were housed on concrete after weaning until all litters in the group had been weaned (approximately 2 weeks spread in age from the oldest to the youngest) and were hand fed twice daily in galvanized metal troughs. This group was moved to pasture as soon as all litters in the group had been weaned. After the first 3 to 5 days, the 56-day litters were allowed to run with their dams on pasture. All pigs were given iron pills at 2 to 4 days of age.

Results

Reproductive Behavior of Sows

Days to First Heat. The mean number of days, the range and the modal number or days required to exhibit the first post-weaning estrus are shown in table 2. The 9.40, 6.25 and 4.0 days required for the 10-day, 21-day and 56-day weaned groups, respectively, differed significantly ($P<0.05$).

Ovulation Rate. Two of the 10 sows in the 10-day weaned group had no corpora lutea at the time of autopsy. Their ovaries contained only follicular cysts 14 mm. in diameter or larger, therefore these two animals were not

included in the ovulation rate study. The three groups did not differ significantly in ovulation rate; however, the probability approached significance ($P \cong 0.06$) (table 2).

Fertilization Rate. The three groups did not differ significantly in percent fertility (table 2). Two sows in the 10-day weaned group that were completely cystic were not included in the fertility data. One sow was omitted from the 21-day group because of pyometra and one from the 56-day group for failure to recover any ova.

TABLE 3. SOW AND LITTER WEIGHT GAINS AND FEED REQUIREMENTS

Weaning age of litter	21 days	56 days
Total no. litters	12	13
Total no. farrowed	98	116
Av. no. pigs farrowed	8.2	8.9
Av. no. pigs at 21 days	6.5	6.7
Av. wt. at 21 days, lb.	11.7	11.6
Av. wt. at 56 days, lb.	29.2	37.5*
Av. daily gain (21 to 56 days), lb.	0.50	0.74*
No. pigs died (21 to 56 days)	2	0
Sow weight, lb.		
Prefarrowing	556	562
At 21 days	500	494
At 56 days	...	479
Feed requirements (21 to 56 days), lb.		
Starter	1127	....
Creep	1576	1035
Sow	....	7964
Feed cost per lb. gain, cents	12	14

* Probability less than 0.05.

Sow and Pig Gains. The pigs from the 10-day weaned group failed to survive under the conditions of this experiment, therefore comparisons for rate and economy of gain were made only between the 21-day and 56-day group. The respective average weights of the 21-day group and the 56-day group differed significantly (29.2 and 37.5 lb. $P < 0.01$) at 56 days of age as did the average daily gain of 0.50 lb. and 0.74 lb. per pig per day from the 21st to the 56th day. Two pigs died in the 21-day group and none in the 56-day group between the 21st and 56th day (table 3).

The 56-day group had an average loss of 15 lb. per sow from the 21st to the 56th day which should be charged against the cost of pig gains for this group. The 5-week delay before marketing these sows occurred on a declining market to decrease the return per pound, thus adding to the cost of pig gains. Conversely, a delay on a rising market would have resulted in a higher return per pound but the loss in total sow weight would still have to be considered.

Feed costs from farrowing to the 21st day were assumed to be the same for the two groups, thus feed costs were figured from the 21st to the 56th

day. The feed required and the calculated feed costs are shown for each group in table 3. These estimates of 12 and 14 cents per pound of gain for the 21-day and 56-day groups, respectively, include only feed costs; therefore, the additional shelter area, forage, and decrease in sow salvage value due to weight loss and market decline would further increase the cost per pound of pig gain in the 56-day weaned group. An estimate of this additional cost was not made.

Discussion

The earlier studies (Grummer—unpublished data) had indicated that weaning at 3 weeks of age need not necessarily decrease rate of gain or increase the costs unduly, provided proper facilities, sanitation and temperature are maintained and the numbers of pigs per group are small (8 to 10 pigs). In the present study the economy of gains for pigs weaned at 3 weeks of age was not in excess of those for pigs remaining on their dams (12 cents per pound for 3-week weaned pigs and 14 cents per pound for 56-day weaned pigs). The inferior rate of gain of the pigs in this study as compared to the earlier observations at this station is attributed almost entirely to the bunching of pigs of varying ages into a single group which resulted in more crowding at the feed trough and in the shelter area.

Post-56-day gains were not observed in the current study. Hanson (1956) reported satisfactory rate and economy of gains for early-weaned pigs but the rate and economy of gains of early-weaned pigs subsequent to 56 days appeared to be inferior to 56-day weaned pigs.

In the present study weaning earlier than 56-days of age resulted in decreased efficiency in sow reproduction; however, costs of pig gains and salvage value of the sows were more favorable for those litters weaned at 3 weeks as compared to weaning at 8 weeks.

The incidence of a post-partum estrus in only 3 of the 34 sows (8.8%) is considerably lower than the 32% and 62.5% reported by Warnick *et al.* (1950). Baker *et al.* (1953) reported post-partum heats in 59% of 29 sows 1 to 3 days after farrowing. Both groups of workers concluded that suckling by the pigs after farrowing had little influence on the frequency of post-partum heats. No explanation is apparent for the discrepancy of the frequency of a post-partum heat in their sows as compared to those of the present study.

That suckling may act as an exogenous stimulator or inhibitor of certain endocrine activity is indicated by the work of Warnick *et al.* (1950) where the average follicular volume was significantly less in post-partum estrous gilts suckled for 10 days than in non-suckled gilts, and the average length of the post-partum estrus was significantly longer in the non-suckled than in the suckled groups. Baker *et al.* (1953) found that suckling for only 2 days exerted no influence on length of the post-partum heat. A majority of the sows studied by Baker *et al.* (1953) were slaughtered on the 21st

day, but some in the latter phases of the study were held to the 31st day. At the time of autopsy only about three-fourths (75.9%) of the sows had exhibited a normal ovulatory estrus. The average number of days from parturition to a fertile estrus was 11.73 for those sows which exhibited heat. It appears safe to conclude that the remaining one-fourth of their sows which had not shown estrus by the time of slaughter at the 21st or 31st day would have increased the mean of 11.73 days appreciably if all sows had been allowed to express an estrus. In their study the pigs were removed either at birth or at two days of age, thus the mean number of days from weaning to a post-weaning estrus for all sows in their study would be appreciably greater than the mean for the sows in the present study that suckled litters to 10 days of age. If this latter conclusion is valid, it appears that as the weaning age of the litter increases from 1 day to 56 days of age a progressively shorter period of time is required for sows to exhibit estrus following weaning of their litters.

A lactation is usually initiated by the processes associated with parturition. The residual endogenous elements of this initiation process appear to be sufficient to maintain lactation and to inhibit ovulatory heats until such a time as the exogenous stimulus of the nursing young will replace it. If so, there must be a period of dual responsibility which gradually transforms to a regime in which continued lactation and heat inhibition is dependent solely on the exogenous stimulus of nursing. In such a case either of these stimuli would be capable of inhibiting an ovulatory heat during lactation, thus removal of the pigs prior to the exhaustion of the endogenous stimulus allows it to function for a time before receding sufficiently to allow estrus to occur. Greater variability in the time required to exhibit estrus in the 10-day and 21-day groups than in the 56-day group indicates that the endogenous stimulus has been exhausted to a greater degree by the 56th day than by either the 10th or 21st day, following farrowing. Thus, removal of the exogenous stimulus (nursing) allowed estrus to occur more uniformly following weaning in the 56-day group of sows.

The occurrence of a complete cystic condition in 2 of the 10 sows, (20%), a reduction in ovulation rate that approached significance ($P \cong 0.06$), and a significantly longer time required to exhibit heat in sows whose pigs had been removed at 10 days of age as compared to 56-day weaning seems to indicate the lack of attainment of the proper hormone output, the proper hormone balance, or the lack of sensitivity of the target organs to the substances required to trigger them. The complexity of this regulating mechanism is not known, but it is known that altering the level of only one hormone (progesterone) will inhibit estrus, increase the incidence of cysts and cause lowered fertility at the first post-treatment estrus (Ulberg, *et al.*, 1951; Baker *et al.*, 1954) but the manner in which these phenomena were effected is not clear.

Slaughter of most of the sows in this study prevented studying their reproductive performance at subsequent farrowings, thus there is no

knowledge of the effects when sows have their litters removed early at several consecutive seasons as compared to removal at the usual 8 weeks of age.

Summary

Thirty-five litters were weaned at either 10, 21, or 56 days of age. Pig and sow feed costs, pig growth rate and sow reproductive behavior were obtained. The days required to exhibit the first post-weaning estrus were 9.4, 6.25, and 4.0 ($P<0.05$) for the 10, 21, and 56 day groups, respectively. Two sows (20%) in the 10-day group had cystic ovaries. The ovulation rate for the 10,- 21,- and 56-day groups was 12.8, 15.2, and 16.6 ova, respectively ($P\cong0.06$). The percent of the recovered ova showing normal cleavage did not differ for the three groups. Pigs weaned at 10 days of age failed to survive under the conditions of this study. Sows whose litters were weaned at 56 days of age lost 15 lb. from the 21st to the 56th day and feed cost per pound of gain in the 21-day weaned pigs did not differ from those weaned at 56 days. Pigs weaned at 21 days of age and held in one large group gained significantly slower from the 21st to the 56th day of age than pigs weaned at 56 days (0.50 and 0.74 lb., respectively, $P<0.05$).

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DIURNAL VARIATIONS IN THE COMPOSITION OF SWINE FECES [1]

D. J. HORVATH,[2] M. E. PETERSON,[3] A. J. CLAWSON,[4] B. E. SHEFFY AND J. K. LOOSLI

Cornell University, Ithaca, New York

THE current interest in studies of diurnal variations in biological material has been critically considered by Cole (1957), who established that "corrected" values from a table of random numbers displayed a cyclic pattern. Despite this interest only one comprehensive analysis (Moore, 1957) of the constituents of feces collected at different hours has been located. Moore showed a diurnal pattern in the constituents of the feces of swine fed twice or three times daily. He concluded that differential rates of passage of various feed fractions through the stomach were an important factor in causing the variation. Bradley *et al.* (1956) using sheep and Oldfield *et al.* (1956) using cattle have noted that fecal nitrogen concentration varies. In the case of cattle, the variation was cyclical. A number of workers have noted that excretion of Cr_2O_3 exhibits a diurnal pattern, but ordinarily the experimental subjects received the indicator either periodially in capsule form or only in the hand-fed concentrate portion of the ration, and therefore cyclical variation would be expected.

Clawson *et al.* (1955) reported that excretion of Cr_2O_3 by pigs self-fed a ration in which the indicator had been thoroughly mixed, regularly varied about 5% between the 10:30 a.m. and 8:30 p.m. samples. Samples taken at 5:30 a.m. and 3:30 p.m. more nearly approximated the levels of Cr_2O_3 calculated for 100% recovery than samples obtained at other times.

Inadequate mixing of the ration or settling of the Cr_2O_3 due to the action of the agitators in a self-feeder would be expected to contribute to random, not cyclical variation. Prior to Moore's study, (1957) the authors had postulated that the higher Cr_2O_3 concentration at 10:30 a.m. was due to the extended digestion time of ingesta in the cecum and the physiologically equivalent portion of the colon during the night when pigs are generally inactive and not defecating. During the day, the frequency of eating and defecating would probably reduce the time ingesta spends in the cecum. Castle and Castle (1956) have shown that feed fed in the morning passed through the tract of pigs in significantly less time than identical feed fed in the evening. In contrast to the material in the cecum, the first material voided each morning presumably has been in the lower colon

[1] The advice of R. S. Dunbar, West Virginia Experiment Station Statistician is acknowledged and appreciated.

[2] Present address, West Virginia University, Morgantown, West Virginia.
[3] Present address, Brigham Young University, Provo, Utah.
[4] Present address, North Carolina State College, Raleigh, North Carolina.

TABLE 1. RATIONS FED TO THE PIGS IN EXPERIMENT II

Ingredients	Basal	Basal plus 3% fat	Basal plus 10% fat
	lb.	lb.	lb.
Corn	75.0	71.3	62.5
Soybean oil meal	14.0	14.7	16.5
Alfalfa	5.0	5.0	5.0
Meat scrap	5.0	5.0	5.0
Fat ("brown grease")	....	3.0	10.0
Salt	0.5	0.5	0.5
Aurofac	0.5	0.5	0.5

and rectum overnight and therefore subjected to less digestion in that interval.

To obtain data on variation in composition, samples of feces from eight pigs in one lot in the study of Clawson *et al.* (1955) and those from four other pigs were analyzed by modified A.O.A.C. methods for nitrogen and ash.

TABLE 2. FECAL COMPOSITION AS PERCENT OF DRY MATTER. (EACH VALUE IS THE AVERAGE OF 4 DAYS' SAMPLES)

Experiment I

Pig	NNOM[a]		Protein		Ash		Cr_2O_3 (mg. per gram feces)	
no.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
1	64.13	66.04	22.13	21.34	13.74	12.64	19.9	18.5
2	62.76	66.00	21.60	20.14	15.64	13.86	26.4	23.1
3	66.97	67.20	21.37	21.24	11.91	11.56	19.0	18.6
4	68.49	68.15	19.26	19.03	12.26	12.82	18.2	18.6
5	67.54	68.21	19.73	19.23	12.79	12.56	18.8	17.3
6	68.92	70.34	17.66	17.02	13.43	12.60	19.8	19.2
7	64.69	65.50	20.75	20.52	14.56	13.98	22.6	22.2
8	65.62	66.81	22.28	21.50	12.78	11.69	18.2	16.5
Mean	66.14	67.28	20.60	20.00	13.39	12.72	20.4	19.2

Experiment II

Pig		NNOM		Protein		Ash	
no.	Diet	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
1	Basal	64.93	67.14	22.03	19.97	13.04	12.89
2	3% fat	62.85	61.84	21.33	22.65	15.82	15.52
3	10% fat	62.28	62.68	20.01	20.03	17.71	17.30
4	10% fat	63.44	63.38	20.81	20.90	15.75	15.72
Mean		63.38	63.76	21.05	20.89	15.58	15.35

[a] Non-nitrogenous organic matter.

Experimental Procedures

The eight self-fed pigs in Experiment I received a diet containing 3% fat, 12.5% alfalfa meal, 69% corn, and 9.5% soybean oil meal. The pigs were roused at 5:30 and 10:30 a.m. and at 3:30 and 8:30 p.m. for 4 days and the feces were collected as voided. The samples were dried at approximately 70° C. for one week. Only the 10:30 a.m. and 8:30 p.m. samples were analyzed for the purposes of this paper.

The pigs in Experiment II had been receiving diets containing 0%, 3%, or 10% additional animal fat ("brown grease"), as noted in table 1, in an experiment by DeCosta (1956). Feces were obtained when the pigs were roused at 8 a.m. and 5 p.m. These samples were dried at 100° C. for not less than 48 hours, ground and stored.

Analyses for nitrogen, ash and dry matter were conducted on duplicate sub-samples.

Results and Discussion

The average values obtained in both experiments are presented in table 2.

In Experiment I statistical analyses of the individual values for ash, protein, and non-nitrogenous organic matter (NNOM) revealed highly significant differences for pigs and hours (table 3).

It is evident that protein and ash varied in the same manner as did Cr_2O_3, whereas NNOM varied inversely with these constituents. The correlation between ash and protein was +.207; that between NNOM and protein was —.534. This trend is in agreement with the findings of Moore (1957). It is tempting to consider that the extended period of microbial digestion afforded material remaining in the upper large intestine overnight leads to the reduced level of NNOM, particularly fiber and other complex carbohydrates. At the same time, the increase in protein may represent an increase in bacterial protein, though intestinal secretions may also be involved, and could perhaps be considered the "cost" of digestion of the NNOM.

The assumption implicit in this reasoning is that the microorganisms are more active in the upper than in the lower portion of the large intestine. Positive evidence for this assumption is lacking. It is also possible that somewhat similar digestion continues in the lower portion of the large intestine, but that the products of digestion are not absorbed to the same extent as is the case in the upper portion. It should be recalled that all values in this paper are considered as percentage of fecal dry matter and are not based on digestibility or recovery.

Moore (1957) stresses the differential rate of passage of materials through the stomach as an explanation of diurnal variations. It would seem that in self-fed animals fluctuations due to this factor would tend to overlap with the increased frequency of eating and thus minimize the variation in composition. Analyses for only two hours of the day do not justify

TABLE 3. STATISTICAL ANALYSES OF DATA FROM EXPERIMENTS I AND II

	Experiment I			Experiment II		
Ash						
Source of variation	DF	MS	F	DF	MS	F
Total	63	.....	.....	31	.....	.....
Pigs	7	8.21	17.85**	3	28.13	51.14**
Days	3	0.89	NS[a]	3	0.28	NS[a]
Hours	1	7.21	15.67**	1	0.41	NS
Pigs x days	21	0.26	NS	9	0.92	NS
Pigs x hours	7	0.98	NS	3	0.06	NS
Days x hours	3	0.55	NS	3	0.92	NS
Residual	21	0.46	.....	9	0.55	.....
Crude protein						
Source of variation	DF	MS	F	DF	MS	F
Total	63	.....	.....	31	.....	.....
Pigs	7	19.20	50.52**	3	5.26	14.61**
Days	3	0.57	NS	3	0.43	NS
Hours	1	5.63	14.82**	1	0.19	NS
Pigs x days	21	0.38	NS	9	0.54	NS
Pigs x hours	7	0.26	NS	3	3.87	10.75**
Days x hours	3	0.35	NS	3	0.53	NS
Residual	21	0.38	.....	9	0.36	.....
Non-nitrogenous organic matter						
Source of variation	DF	MS	F	DF	MS	F
Total	63	.....	.....	31	.....	.....
Pigs	7	26.99	45.75**	3	23.36	43.26**
Days	3	2.49	4.22*	3	0.32	NS
Hours	1	20.99	35.58**	1	0.71	NS
Pigs x days	21	2.41	4.08**	9	1.79	NS($P<.10$)
Pigs x hours	7	1.42	NS	3	3.81	7.06**
Days x hours	3	0.44	NS	3	2.44	4.33*
Residual	21	0.59	.....	9	0.54	.....

** $P<.01$.
* $P<.05$.
[a] Non-significant.

assuming that such over-lap failed to occur, but to the extent that the protein and ash follow closely the four daily Cr_2O_3 values of Clawson *et al.* (1955), this is the implication.

Differences due to differential rate of passage through the stomach might also be expected to be reduced by the mixing action of the upper large intestine. Certainly both factors could be operating simultaneously,

and in light of the detailed evidence presented by Moore, differential rate of passage cannot be overlooked. Briefly, Moore's fecal composition data show rather sharp slopes in the early morning which may support the role of microorganisms, but feeding three times daily resulted in three peaks which supports his contention, as does his slaughter study.

The implications of these observations with reference to the validity of "grab-sampling" will vary with the constituent being studied and the degree of accuracy demanded by the problem. Firstly, the relative change was not the same for all principles. Secondly, those that varied in the same direction as the indicator would be less in error than those that varied inversely with the indicator (*e.g.*, NNOM). Individual variation will also be critical, since as emphasized by the pig x hour interaction seen in the case of NNOM in Experiment I, not all pigs' cycles are in phase. Moore (1958) has considered these problems in detail.

In Experiment II statistical analysis revealed that in no case was the mean square for "hours" significant (table 3).

This lack of signifiance is not in agreement with the results of Experiment I, nor with the findings of Moore. One reason is the greater variation in results from day to day as reflected in the pig x hour interactions The trend was actually reversed on one day, possibly because of the light and noise in the barn at night when farrowing sows were attended by the herdsman. If the pigs had been aroused, defecation would have been likely and would, in our opinion, have altered the cycle. Admittedly more frequent sampling and a deliberate control *vs.* night-lighting treatment would have been desirable. In addition there were fewer degrees of freedom in the residual term than in Experiment I. It should be noted that one individual in Experiment I consistently showed reversal of the usual pattern.

Further, the choice of sampling time seems, from the Cr_2O_3 data of Clawson *et al.* (1955), to be less likely to reveal differences than the 10:30 a.m. and the 8:30 p.m. samples used in Experiment I.

Summary

Evidence has been presented to substantiate the nature of the diurnal variation in fecal Cr_2O_3 values reported for self-fed pigs by Clawson *et al.* (1955).

Cr_2O_3 concentration was greater in the morning than evening and was associated with greater concentrations of nitrogen and ash. Non-nitrogenous organic matter was present in greater concentration in the afternoon or evening samples than in those obtained in the morning.

Possible reasons for the diurnal variation in fecal composition are considered to be the differences in time of digestion in the lower tract and/or differences in rate of passage of various feed fractions through the stomach.

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PERFORMANCE OF BARROW AND GILT LITTERMATE PAIRS AT THE OHIO SWINE EVALUATION STATION[1]

W. H. BRUNER,[2] VERN R. CAHILL, W. L. ROBISON,[3] AND RICHARD F. WILSON[4]

Ohio State University,[5] Columbus, and The Ohio Agricultural Experiment Station, Wooster

THIS study originated as a result of observations made on Ohio Swine Evaluation Station littermate barrows and gilts. In the majority of instances the barrow reached 210 lb. live weight at a younger age than the gilt. Likewise, carcass measurements, lean cuts of carcass weight and/or primal cuts of live weight appeared to be different for barrows than for gilts. Self, Bray and Reierson (1957) reported gilts had larger loin eye measurements than barrows.

The importance of rate of gain and carcass performance is well recognized in the several evaluation and certification meat-type programs. There appeared to be a need, however, for more information concerning the rate of gain and carcass value of littermate barrows and gilts.

Materials and Methods

The Ohio Swine Improvement Program was inaugurated in 1948 and is administered by the Swine Improvement Association of Ohio. Its principal purpose is to aid in recognition and selection of breeding stock from Ohio herds that will improve the efficiency of production and the market value of hogs. In 1954 the Ohio State University constructed a Swine Evaluation Station.

Participation by breeders is by voluntary nomination of litters that are eligible for breed registry and are free of visible heritable defects. Litters with a minimum of eight pigs must meet their respective breed association's weight standards. A representative pair, a barrow and a gilt, if possible, are selected for entry into the Station by a committee composed of the breeder and either a county agent, or a vocational agricultural teacher, or a county agent appointee.

The Swine Evaluation Station, the equipment, its operation and supervision are provided by the Ohio State University in cooperation with the Ohio Agricultural Experiment Station. Table 1 shows the composition of

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[2] Also a member of the Agricultural Economics Department, Ohio State University.

[3] In charge of swine investigations at Ohio Agricultural Experiment Station, Wooster, until his death in 1956.

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[5] Animal Science Department.

feeds fed at the Station. Slaughter test work is done by the Meats Laboratory of the Animal Science Department, Ohio State University. The method followed in carcass cutting is described in the Proceedings of the 5th Annual Reciprocal Meat Conference (1952). Pairs of pigs, starting at 63 days of age, are fed together and are taken off test individually as each pig reaches approximately 210 lb. live weight.

TABLE 1. COMPOSITION OF FEEDS FED AT THE OHIO SWINE EVALUATION STATION

Ingredients	Feed no. 1 [a]	Feed no. 2 [b]
Coarsely ground shelled yellow corn, lb.	1303.0	1439.0
Finely ground oats, lb.	250.0	200.0
Meat scraps (55% C.P.), lb.	144.0	102.0
Soybean oil meal (44% C.P., solvent processed), lb.	164.0	116.0
Dehydrated alfalfa meal (17% C.P. or more), lb.	100.0	100.0
B-vitamins, antibiotic and yeast premix,[c] lb.	17.2	17.2
Mineral mixture, lb.	22.0	26.0
	2000.2	2000.2

[a] Fed from time the pair of pigs started in the Station until they averaged approximately 120 lb. This feed was calculated to contain 15.5% crude protein.

[b] Fed from the time the pigs averaged approximately 120 lb. until they individually reached 210 lb. This feed was calculated to contain 13.6% crude protein.

[c] The premix furnished per pound of total feed: 2.5 mg. riboflavin; 5.0 mg. calcium pantothenate; 11.25 mg. niacin; 12.5 mg. choline chloride; 0.31 mg. pyridoxine; 0.005 mg. B_{12}; 5 mg. chlortetracycline; and 408.5 I.U. of vitamin A.

The backfat measurement reported represents the average of three carcass measurements, the first being taken opposite the leading edge of the first rib, the second opposite the last rib and the third opposite the last lumbar. Carcass length was the distance from the junction of the neckbone and the first rib to the anterior crest of the aitch bone at its lowest point as the chilled carcass hung on the rail.

Loin eye measurement was secured by tracing only the longissimus dorsi muscle of the chilled loin between the tenth and eleventh rib. The K-L planimeter was used to determine the square inches of the loin eye from the tracing. The primal cuts consisted of the skinned ham, the trimmed loin, the New York style shoulder and the belly. The adjusted live weight used was the live weight at slaughter adjusted by the difference between the weight of the stomach, the intestines and their contents and a 20-pound standard.

The statistical analyses were conducted according to Snedecor (1946).

Results and Discussion

Five seasons of data (1954 fall through 1956 fall) for 385 full sib pairs (barrow and gilt) are reported in tables 2 and 3.

TABLE 2. THE MEANS AND STANDARD DEVIATIONS FOR THE FACTORS STUDIED[a]

Items	Means[b]		Standard deviations	
	Barrows	Gilts	Barrows	Gilts
Number of animals	385	385	.....	.....
Sixty-three day weight, lb.	40.34	40.55	7.89	7.37
Age at 210 lb., days	160.10	167.10	11.86	12.81
Daily gain, lb.	1.77	1.64	0.18	0.18
Backfat thickness, in.	1.66	1.56	0.16	0.16
Carcass length, in.	29.76	30.17	1.08	1.12
Loin eye area, sq. in.	3.46	3.97	0.55	0.57
Trimmed loin weight, lb.	22.25	23.33	1.54	1.57
Skinned ham weight, lb.	27.49	29.13	1.70	1.87
Lean cuts of chilled carcass weight, %	49.28	51.58	2.33	2.42
Primal cuts of adjusted live weight, %	47.96	49.07	1.54	1.61

[a] Average slaughter weight was 206.6 lb. for the barrows and 206.0 lb. for the gilts.
[b] Except for the differences in 63-day weights, all differences between barrow and gilt means are statistically significant at the 1% level.

In table 2 the means and standard deviations of the various traits studied are listed. The most striking features noted were (1) barrows reached 210 lb. weight at 160.1 days of age compared to 167.1 days for gilts, (2) the barrow carcasses yielded 2.30% less lean cuts than the gilt carcasses, and (3) a difference of 0.41 in. in carcass length between barrows and gilts, and (4) a large difference of 0.51 sq. in. in loin eye area between the two sexes. All differences between barrows and gilts were significantly different at the 1% level with the exception of 63-day weights.

The standard deviations for each trait compared are quite similar, but considerable variation is indicated by the relatively large values except for percent primal cuts of adjusted live weight.

The correlations between the various traits are listed in table 3. The

TABLE 3. CORRELATION BETWEEN CARCASS CHARACTERISTICS

Items	Barrows	Gilts
Backfat and loin eye measurement	−.21**	−.19**
Backfat and percent primal cuts	−.44**	−.48**
Carcass length and loin eye measurements	−.13*	−.15**
Carcass length and percent primal cuts	.18**	.17**
Carcass length and percent lean cuts	.24**	.31**
Loin eye and percent primal cuts	.57**	.56**
Loin eye and percent lean cuts	.55**	.52**
Backfat and carcass length	−.27**	−.41**
Loin eye measurement and pounds of loin	.61**	.54**
Loin eye measurement and pounds of ham	.60**	.54**

* Significant at the 5% level.
** Significant at the 1% level.

highest correlation is 0.61 for barrows comparing relationship of loin eye measurement and pounds of loin. The next highest correlation is the relationship of loin eye measurement and pounds of ham. Two of the lowest correlations for both barrows and gilts are between carcass length and loin eye, and carcass length and percent primal cuts.

Because of the distinct differences in performance of littermate barrows and gilts it appears advisable to include both in a selection program for the evaluation of prospective breeding stock.

Summary

A study was made of certain differences observed in 385 littermate barrow and gilt pairs tested in the Ohio Swine Evaluation Station. The barrows gained faster than the gilts. The gilts produced leaner carcasses than the barrows, based on percent of lean cuts of carcass weight, backfat thickness and loin eye measurement, and weight of loin and ham.

Loin eye area was correlated positively and backfat measurements negatively with primal cut yield of live weight in both barrows and gilts.

Pounds of ham and pounds of loin in both barrows and gilts were positively correlated with loin eye measurements. Backfat and loin eye measurement were more closely correlated than carcass length with lean cuts of carcass weight and/or primal cuts of live weight.

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THE EFFECT OF EXOGENOUS PROGESTERONE AND LEVEL OF FEEDING ON PRENATAL SURVIVAL IN GILTS [1]

C. E. HAINES, A. C. WARNICK AND H. D. WALLACE

Florida Agricultural Experiment Station,[2] Gainesville

THE prenatal death rate in swine is known to be a very important factor in determining subsequent litter size. Many different theories have been tested in an attempt to reduce early embryonic mortality in swine and other litter bearing animals. A few studies have indicated that there is a relationship between progesterone production in the body and the normal implantation and development of embryos.

Kimura and Cornwell (1938) determined that the corpora lutea of sows in different stages of pregnancy contained varying amounts of progesterone. They reported a sharp rise in progesterone content during pregnancy until the 20th day and the content remained high until the 105th day of pregnancy. A study by Glasgow and co-workers (1951) found that there was a significant correlation between the concentration of progesterone excretion products and the percentage of embryonic survival.

In a study by Sammelwitz *et al.* (1956) injections of progesterone were not beneficial in reducing early embryonic mortality in gilts. The gilts were treated with 50, 100, 200 or 400 mg. of progesterone daily for 26 days after breeding. The higher dosages appeared more detrimental than the lower doses, but all of the treated gilts had higher rates of embryonic mortality than the control gilts. Hammond (1957) states that progesterone has an inhibitory effect on uterine contractions and suggests that this action should make progesterone beneficial for implantation.

It would appear that a correct exogenous dosage of progesterone in the early stages of pregnancy might aid implantation and the development of the embryos until the corpora lutea could assume this responsibility. In an attempt to evaluate this theory further, a study was conducted to determine the effects of supplementary progesterone on early embryonic survival in gilts on two levels of energy intake.

Materials and Methods

Eighty-five young Duroc Jersey gilts, weighing about 100 lb., all sired by two boars, were full-fed a growing ration on pasture and examined daily for the occurrence of first estrus. Fifty-four of these gilts were born in January and February and the remaining 31 in April. The age and weight of each gilt at first estrus was recorded and the gilts were continued on full feed until

[1] Florida Agricultural Experiment Station Journal Series, No. 741.

[2] Department of Animal Husbandry and Nutrition. Progesterone was generously supplied by International Hormones, Brooklyn, New York.

TABLE 1. THE COMPOSITION OF TEST RATIONS (PERCENT)

Ingredients	Full-fed ration	Limited-fed ration
Ground corn	50	16
Ground oats	23	0
Alfalfa leaf meal	10	20
Soybean oil meal	10	44
Meat and bone scraps	5	17
Limestone	1	1
Steamed bone meal	1	1
Trace mineralized salt	0.53	1.06
Aurofac 2-A (Lederle)	0.3	0.6
Total	100.83	100.66

the second estrual period. The gilts were bred once during the second estrus and alternate females were switched to a limited system of feeding. The limited-fed ration contained one-half of the energy intake of the full-fed ration, but contained equivalent amounts of protein, vitamins and minerals. The limited-fed gilts were provided with one-half of the total amount of feed that the full-fed gilts consumed daily. The compositions of these two rations are compared in table 1. All gilts were maintained on pasture during the experiment.

Beginning with the third day after breeding, one-half of the gilts in both the limited and full-fed groups were injected intramuscularly with 25 mg. of progesterone in corn oil, every other day. Injections were administered in the neck region, on alternate sides, with an 18 gauge needle. Injections terminated on the 25th day of gestation and all gilts remained on their respective postbreeding rations until sacrificed or allowed to farrow. Approximately one-third of the gilts in each ration and hormone group were sacrificed at 25 days postbreeding, another one-third at 40 days postbreeding and the remaining females were allowed to farrow. A diagrammatic scheme of the experimental design and the number of gilts in each sub-group is presented in table 2. The gilts were assigned to the various sub-groups at the time of breeding. Although 85 gilts provided data on puberty, survival data were obtained from only 80 females.

TABLE 2. THE EXPERIMENTAL DESIGN, NUMBER OF GILTS AND PERIOD OF DATA COLLECTION

	Full-fed gilts		Limited-fed gilts	
Gestation length	Progesterone	Control	Progesterone	Control
25 days	6	7	6	6
40 days	7	7	7	7
Term	7	7	6	7
Total	20	21	19	20

At the time of sacrifice (25 or 40 days of gestation), the entire reproductive tract was obtained and examined. Embryonic survival rates were calculated by comparing the number of corpora lutea on the ovaries with the number of normal embryos present in the uterus of gilts slaughtered at 25 and 40 days postbreeding. The embryonic survival rates for the gilts that farrowed were calculated by comparing the average ovulation rate of all 25- and 40-day gilts to the number of live pigs farrowed by each gilt. An analysis of variance test, as described by Snedecor (1946), was conducted on the differences among groups for the various factors studied.

A committee of three rated the 54 gilts born in January and February on feminine characteristics about the head and face. Ratings were made when the gilts averaged about 200 days of age. The average of the three ratings was assigned to the individual gilt and correlated with her ovulation rate and age at puberty. The 31 gilts that were born in April were not rated for femininity.

Results

Seasonal Differences in Puberty. The 54 gilts farrowed during January and February averaged 229 $\pm$ 24 days of age and 246 $\pm$ 11 lb. at puberty. Gilts farrowed during April averaged 206 $\pm$ 33 days of age and 214 $\pm$ 22 lb. at puberty. Thus, gilts farrowed later in the season averaged 23 days younger and 32 lb. lighter at puberty than those farrowed two months earlier. These differences, in both age and weight at puberty, were statistically significant ($P<0.01$). Since all gilts were closely related and on good quality grass pasture at least 3 months before reaching puberty, these differences appear to be due to season or climatic factors, rather than to levels of feeding. This difference in sexual development caused by seasonal factors is similar to those reported by Wiggins *et al.* (1950) and Robertson *et al.* (1951) in a temperate climate.

Femininity Ratings. Femininity ratings, assigned when the gilts were approximately 200 days old, were significantly correlated with age at puberty ($r=0.32$), indicating that the more feminine gilts attained puberty at a younger age. These data differ from those of Rio *et al.* (1955), who reported no association between femininity ratings of the head and age at puberty. The correlation between femininity and ovulation rate at the second heat was positive but not significant ($r=0.022$). Fowler and Robertson (1954) reported a significant positive correlation between femininity ratings and ovulation.

Twenty-five Days of Gestation. The ovulation rate differed slightly among groups but this was probably due to sampling and was not significant. The average number of normal embryos varied from 10.2 in control gilts on limited feed to 13.6 embryos for control gilts on full feed (table 3). However, the exogenous progesterone appeared to reduce embryonic mortality on the limited ration but not on the full-fed ration. The differences in embryonic survival at this stage, due to either ration or hormone, were not significant at the 5% level.

TABLE 3. THE EFFECT OF EXOGENOUS PROGESTERONE AND RATION ON EMBRYONIC SURVIVAL TO 25 DAYS OF GESTATION

Ration	Full-fed		Limited-fed	
Hormone treatment	Progesterone	Control	Progesterone	Control
No. of gilts	6	7	6	6
No. corpora lutea	13.7	15.0	13.8	14.7
No. normal embryos	10.3	13.6	12.7	10.2
Percent survival	76.5	90.7	91.0	67.1

Forty Days of Gestation. The average ovulation rate was similar for all four groups, varying from 12.7 to 13.9 (table 4). The number of normal embryos varied from 9.0 in gilts receiving progesterone to 11.3 embryos in gilts with no progesterone on the full-fed ration.

Exogenous progesterone decreased embryonic survival rate on both rations but the decrease was greater in the full-fed group. Ration and hormone effects were not statistically significant at the 5% level.

Females Allowed to Farrow. Three of the 27 gilts retained for farrowing did not farrow any live pigs, but each was in a different experimental group. Therefore, failure to farrow live pigs was probably not associated with ration or hormone treatment.

Litter size varied from 0 to 12 live pigs per gilt for all groups. The average number of live pigs within sub-group varied from 5.0 for limited-fed gilts with progesterone to 8.1 for full-fed gilts receiving no progesterone (table 5). Percent survival of live pigs was based on the average number of corpora lutea for all gilts slaughtered at 25 and 40 days of gestation. The average percentage survival varied from 36.5 for limited-fed gilts receiving progesterone to 59.4 for full-fed gilts receiving progesterone. Supplemental progesterone appeared to decrease slightly the prenatal mortality on the full-fed ration, while it increased mortality on the limited-fed ration, as indicated in table 5. However, differences in prenatal survival due to ration and hormone treatments were not siginficant at the 5% level.

Comparison of Embryonic Survival During Gestation. Since differences

TABLE 4. THE EFFECT OF EXOGENOUS PROGESTERONE AND RATION ON EMBRYONIC SURVIVAL TO 40 DAYS OF GESTATION

Ration	Full-fed		Limited-fed	
Hormone treatment	Progesterone	Control	Progesterone	Control
No. of gilts	7	7	7	7
No. corpora lutea	13.0	13.1	13.9	12.7
No. normal embryos	9.0	11.3	10.0	9.7
Percent survival	68.6	86.0	72.4	77.4

TABLE 5. THE EFFECT OF EXOGENOUS PROGESTERONE AND RATION ON EMBRYONIC SURVIVAL BETWEEN OVULATION AND FARROWING

Ration	Full-fed		Limited-fed	
Hormone treatment	Progesterone	Control	Progesterone	Control
No. of gilts	7	7	6	7
No. corpora lutea [a]	13.7	13.7	13.7	13.7
No. live pigs born	8.1	6.9	5.0	7.4
Percent survival	59.4	50.1	36.5	54.2

[a] Number of corpora lutea based on average for gilts slaughtered at 25 and 40 days.

between ration and hormone treatments were not statistically significant, the data from gilts were combined to compare the prenatal survival rate at three stages of gestation. The percentage survival estimates at the three stages of gestation, shown in table 6, indicate a decrease from 81.7% at 25 days to 50.6% at term. Thus, the loss of embryos, or fetuses, was 18.3% from breeding to 25 days, 5.6% from 25 to 40 days and 25.5% from 40 days to term. These differences between survival rates for the three stages were statistically significant.

Statistical Analysis of Data. The analysis of survival rate data showed highly significant differences due to stage of gestation, but not for other main factors studied (table 7). The significant interaction of hormone, ration and stage of gestation is due to an apparent beneficial effect of progesterone in the full-fed ration and not in the limited ration.

Discussion. The results of this study are similar to those of Sammelwitz *et al.* (1956), who found no advantage for progesterone injections in reducing embryonic death in swine. The dosage in the present experiment was 12.5 mg. daily, compared to 50 to 400 mg. in the study by Sammelwitz *et al.* (1956) where no embryos survived at the higher dosages of progesterone. Possibly death may be caused by an imbalance of estrogen and progesterone rather than a deficiency of endogenous progesterone during early pregnancy.

Embryonic survival rates at the three stages of gestation were not altered when gilts were switched to limited feeding from full feeding at the time of breeding. Self *et al.* (1955) reported less embryonic survival at 25 days of gestation when gilts were changed from a full feed to a

TABLE 6. COMBINED EMBRYONIC SURVIVAL RATES AT THREE STAGES OF GESTATION

Stage of gestation	No. of gilts	Corpora lutea	Embryos or live pigs	Percent survival
25 days	25	14.3	11.8	81.7
40 days	28	13.2	10.0	76.1
Term	27	13.7	6.9	50.6

limited ration at breeding. Thus, the results of this study, as well as those of Self, indicate no beneficial effects on embryonic survival due to changing from full to limited feeding at time of breeding.

The overall embryonic survival at 25 days of pregnancy in this study of 81.7% was slightly higher than the 65% reported by Squires *et al.*

TABLE 7. ANALYSIS OF VARIANCE FOR EMBRYONIC SURVIVAL RATES IN GILTS

Source of variation	D/F	Mean squares
Hormone	1	266.0
Ration	1	543.3
Gestation stage	2	7317.5**
Hormone x Ration	1	368.5
Hormone x Gestation stage	2	394.7
Ration x Gestation stage	2	85.5
Hormone x Ration x Gestation stage	2	1711.6*
Error	68	488.9

* $P<0.05$.
** $P<0.01$.

(1952), the 72% by Burger (1952), the 74% by Sorensen and Gossett (1956) and 55% by Robertson *et al.* (1951). The percent survival in this investigation at 40 days of gestation was 76.1%, or a loss of 5.6% between 25 and 40 days. This loss compares closely to that found by Sorensen and Gossett (1956) at 40 days. The percentage survival from breeding to term of 50.9 in our investigation agrees closely to the 54% found by Squires *et al.* (1952) and the 50% reported by Wilson *et al.* (1949). The 62.2 and 75.1% survival rates reported for the pregnancy period by Robertson *et al.* (1950, 1951) is somewhat higher than those of this experiment.

Summary

Eighty-five gilts sired by two boars and weighing approximately 100 lb. were full-fed a growing ration until the second heat. All gilts were bred to fertile boars at the second estrus and one-half of them continued on the full-fed ration. The other gilts were fed a ration that contributed approximately 50% of the energy of the full-fed ration but equivalent quantities of protein vitamins and minerals on pasture. One-half of the gilts on each ration treatment were given 25 mg. of progesterone intramuscularly every other day from the third to the 25th day of gestation. One-third of each sub-group was slaughtered 25 days postbreeding, one-third 40 days after breeding and the remainder were allowed to farrow. The number of normal embryos, live pigs and percentage of embryonic survival were compared between ration and hormone treatments.

Neither the ration used nor the hormone treatment had a significant

influence on the number of embryos, live pigs or embryonic survival. However, the stage of gestation did have a significant influence on embryonic survival.

At 25 days, 40 days and term, there were 11.8, 10.0 and 6.9 embryos or live pigs, respectively. The estimated survival rates at these same stages were 81.7, 76.1 and 50.6%, respectively. Therefore, 25 mg. of exogenous progesterone every other day during the first 25 days of gestation, and/or reducing the energy intake at breeding, did not reduce embryonic mortality.

Gilts farrowed in January and February averaged 229 ± 24 days of age and 246 ± 11 lb. at puberty. Gilts farrowed during April averaged 206 ± 33 days of age and weighed 214 ± 22 lb. at puberty. These differences for both age and weight at puberty due to season or climatic factors were statistically significant ($P<0.01$).

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THE RELATIONSHIP OF DIETARY CALCIUM TO ZINC METABOLISM IN PIGS [1]

H. W. Newland, D. E. Ullrey, J. A. Hoefer and R. W. Luecke [2, 3]

Michigan State University, East Lansing

HIGH dietary levels of calcium have been associated with parakeratosis in swine (Tucker and Salmon, 1955; Luecke *et al.*, 1956). In these experiments it was found that the dermatosis and poor growth could be counteracted by the addition of a small amount (50–100 p.p.m.) of zinc to the ration.

The authors have repeatedly observed that one of the first reactions of pigs fed a high calcium ration was a marked loss of appetite followed by a rapid return to normal when the ration was supplemented with zinc. It therefore seemed desirable to study the calcium-zinc interrelationship in pigs maintained on a controlled energy intake while varying the dietary levels of calcium and zinc.

Materials and Methods

Weanling Hampshire pigs were housed individually in wooden pens, bedded with wood shavings, and fed and watered (distilled H_2O) in wooden troughs.

The basal ration (lot 1) consisted of the following: corn, 75.9%; soybean oil meal, 20.0%; fish meal, 2.0%; limestone, 1.1%; dicalcium phosphate, 0.5%; trace mineral salt, 0.5%; vitamin A and D carrier to supply 1,408 and 200 U.S.P. units of vitamins A and D, respectively, per pound of ration; and a B-vitamin supplement (Merck's No. 58C) added at the rate of 2 lb. per ton of ration.

The calcium and phosphorus levels were raised in rations 2 and 3 for lots 2 and 3 by replacing corn with an equal weight of dicalcium phosphate. $ZnCO_3$ was used to increase the zinc content of the ration for lot 3. By analysis, rations 1, 2 and 3 contained 0.64, 1.19, and 1.23% of calcium; 0.54, 0.93 and 0.96% of phosphorus; and 30, 33, and 94 p.p.m. of zinc, respectively.

Experiment I. Nine pigs (barrows and gilts) were allotted at random into three trios. Each member of a trio received either ration 1, 2, or 3, and feed consumption was restricted to the level of that member which consumed the least. Weight gains, feed efficiency, and incidence of dermatosis were the only data collected. This experiment was terminated at the end of 6 weeks.

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[2] Departments of Animal Husbandry and Agricultural Chemistry.

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Experiment II. Three trios of castrated male pigs were used, each trio consisting of littermates. Each member of a trio received either ration 1, 2, or 3, and feed consumption was equalized as described in Experiment I.

After one week on the experimental diets, the pigs were given a single intravenous injection of Zn^{65} as $ZnCl_2$ in aqueous solution at the rate of 10 microcuries per kilogram of body weight. They were then placed in metabolism units similar to those described by Hansard (1951) for quantitative collection of urine and feces. In a preliminary study it was found that the Zn^{65} activities of the blood and feces reached equilibrium in about 7 days and were approximately parallel thereafter. Therefore, blood, urine and feces collections for endogenous zinc determinations were made for 5 days, between the 8th and 13th day post-injection.

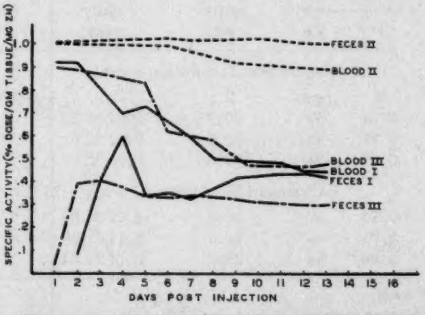

Figure 1. Zinc equilibration of blood and feces post Zn^{65} injection I.V. Trio 2 (feces specific activity × 10).

That this procedure was justifiable is substantiated in the data presented in Figure 1. It can be seen that the specific activity of the blood and fecal zinc had reached a plateau after 7 days post-injection. Visek *et al.* (1953) used this "steady period" of blood and fecal specific activities in their determination of endogenous calcium excretion in cattle. The method for calculating endogenous fecal zinc was patterned after that presented by these workers.

At the end of the 13-day collection period the pigs were killed by exsanguination and certain tissues sampled for analysis.

Urine and feces were wet-ashed; all tissues were dry-ashed, dissolved in a weak acid solution and brought to a known volume with water. Radioassays were made from an aliquot of the liquid sample, a scintillation counter being used in conjunction with a standard scaler. Calcium and phosphorus determinations were made by A.O.A.C. methods (1955), and zinc determinations by the dithiazone procedure described by Benne (1955).

Statistical analyses were performed according to the methods described by Snedecor (1946).

Results and Discussion

Weight Gains; Feed Efficiency; Dermatosis. Data relative to weight gains, feed efficiency and the incidence of dermatosis are presented in table 1. In Experiment I, it was observed that the feed intake of the high-calcium, low-zinc pigs (lot 2) decreased steadily, and after 4 or 5 weeks,

TABLE 1. EFFECT OF TREATMENT ON WEIGHT GAINS, FEED EFFICIENCY AND INCIDENCE OF DERMATOSIS

Lot no.	Dietary Ca	Dietary P	Dietary Zn	Av. initial wt.	Av. daily gain[a]	Feed per lb. gain	No. with dermatosis
			Experiment I; duration 6 weeks				
	%	%	ppm	lb.	lb.	lb.	
1	0.64	0.54	30	20.7	0.17±.12	5.35	1
2	1.19	0.93	33	22.0	0.02±.01	57.00	3[b]
3	1.23	0.96	94	22.0	0.29±.01	3.24	0
			Experiment II; duration 3 weeks				
1	0.64	0.54	30	31.3	0.22±.09	5.07	0
2	1.19	0.93	33	31.3	0.11±.08	8.83	3[c]
3	1.23	0.96	94	28.3	0.27±.04	3.74	0

[a] Mean±standard error.
[b] Severe dermatosis.
[c] Rough hair coats, but only one showed typical lesions.

feed consumption was less than a pound per pig per day. Since uniform feed intake was desired for the balance study, the second experiment was limited to 3 weeks. Even with this precaution, feed intake of the lot-2 pigs averaged only 0.8 lb. per day during the third week. High dietary calcium depressed growth, produced poor feed efficiency and dermatosis (lot 2). Supplementary zinc (lot 3) effectively counteracted these conditions. However, daily gains of the control (lot 1) and zinc-supplemented (lot 3) pigs were considerably below normal and reflected the low energy intake dictated by lot 2.

The symptoms exhibited by the pigs in lot 2 apparently were a consequence of zinc deficiency. It is of interest that in experiments employing synthetic diets extremely low in zinc, the deficiency caused a thickening and hyperkeratinization of the skin in rats (Follis, 1941), skin lesions in mice (Nishimura, 1953) decreased feed efficiency in the rat (Stirn, 1935), decreased absorption of carbohydrates and protein in the rat (Hove *et al.*, 1937), and reddish, scabby skin lesions in pigs (Beardsley and Forbes, 1957). These symptoms are similar to those of the high-calcium, low-zinc pigs reported here.

Zinc Metabolism. The determination of endogenous fecal zinc is based on the assumption that the endogenous zinc reaching the feces will have the same specific activity as the zinc in the blood plasma. For this determination to have meaning, the plasma specific activity must also be in equilibrium with the specific activity of the metabolically active tissues. One of these tissues, the muscle, was examined. Gluteal muscle biopsies

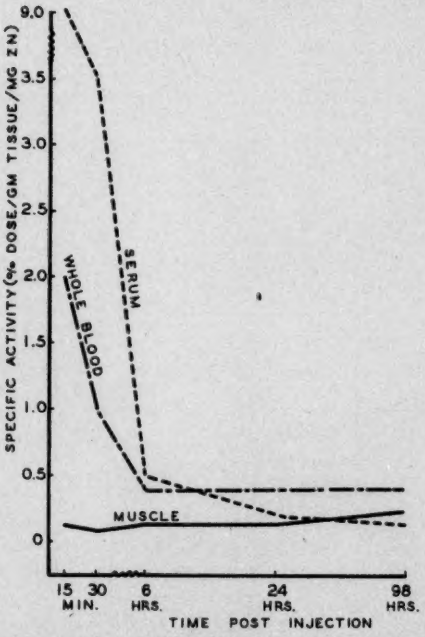

Figure 2. Zinc equilibration of blood and muscle post Zn^{65} injection I.V.

and blood were obtained from two normal pigs at periodic intervals after an intravenous injection of Zn^{65}. Radiozinc and total zinc assays revealed that equilibrium was reached in approximately 24 hours (Figure 2).

Another preliminary study established that the major fecal excretion of intravenously injected Zn^{65} occurred about 24 hours after injection. Hansard *et al.* (1952) using Ca^{45} reported similar results in cattle. Therefore, the specific activity of the feces on any one day was compared to the specific activity of the blood on the previous day.

Daily zinc intake and fecal zinc excretion are presented in table 2. A significantly greater portion of the fecal zinc was derived from endogenous sources in pigs which were maintained on a high-calcium diet unsupple-

TABLE 2. DAILY ZINC INTAKE AND FECAL ZINC EXCRETION. EXPERIMENT II[a]

Group	Wt. at sacrifice	Daily zinc intake[b]	Daily fecal zinc[b]	Daily endogenous fecal zinc	
				Quantity[b]	% of total fecal zinc[c]
	lb.	mg.	mg.	mg.	%
Trio 1					
Lot 1	36	20.78	19.23	0.84	5.02±0.62
Lot 2	38	18.23	14.36	1.19	9.04±0.90**
Lot 3	41	52.20	54.68	1.32	2.57±0.27
Trio 2					
Lot 1	39	8.47	9.44	0.79	8.87±0.78
Lot 2	33	6.87	7.54	1.07	14.73±1.57**
Lot 3	35	28.24	23.18	1.29	5.71±0.22
Trio 3					
Lot 1	32	9.84	12.26	0.59	5.36±0.87
Lot 2	30	11.65	10.50	0.77	8.18±1.03**
Lot 3	28	27.36*	24.86	1.05	4.80±0.68

[a] Feed intake equalized within each trio.
[b] Mean of 5 daily observations.
[c] Mean of 5 daily percentages±standard error.
** Significantly greater ($P<0.01$) than lots 1 and 3.

mented with zinc (lot 2). Total fecal zinc quite closely paralleled zinc intake.

The total zinc content and specific activity of the blood, feces, liver, and kidneys are presented in table 3. The specific activity of the feces and liver was significantly higher for lot 2 than lot 1 or 3, and there was the same trend for blood and kidneys. The concentration of total zinc in

TABLE 3. EFFECT OF TREATMENT ON ZINC CONTENT AND SPECIFIC ACTIVITY OF CERTAIN TISSUES. EXPERIMENT II[a]

Lot no.	Whole blood[b]		Feces[b]		Liver		Kidney	
	Zinc	Specific activity[c]	Zinc	Specific activity	Zinc	Specific activity	Zinc	Specific activity
	ppm		ppm		ppm		ppm	
1	2.59	0.567	248	0.0341	34.8	0.442	23.7	0.366
2	2.70	.769	184	.0807*	25.9	.787**	26.2	.438
3	3.31	.414	500	.0189	38.6	.344	23.6	.348

[a] Three pigs per lot.
[b] Average values for 5-day collection period.
[c] Expressed as % dose/g. of tissue/mg. of total zinc.
* Significantly higher ($P<.05$) than lots 1 and 3.
** Significantly higher ($P<.01$) than lots 1 and 3.

the blood, liver and kidneys was not significantly affected by the dietary calcium or zinc levels. Lewis *et al.* (1956) found that increased levels of dietary zinc or calcium increased the zinc concentration of liver. In the experiment reported here, the animals received the experimental diets for only 3 weeks, which may have been insufficient time for significant changes in tissue concentration to develop.

The pigs in all lots were in positive calcium and phosphorus balance, and the relative amounts excreted via urine or feces were not altered.

Serum alkaline phosphatase values were determined weekly for each pig during Experiment II. The mean Bodansky units for lots 1, 2, and 3 were 1.87 ± 0.49 (SE), 0.76 ± 0.20, and 3.08 ± 0.41, respectively. The values were significantly ($P<.01$) lower for lot 2 than for lots 1 and 3.

The increased specific activity of tissues and the greater proportion of fecal zinc derived from endogenous sources suggested a higher rate of zinc metabolism in pigs receiving high-calcium diets unsupplemented with zinc.

Summary

The effect of various levels of dietary zinc and calcium upon zinc metabolism in weanling pigs was studied. Even though energy intake was equalized between treatments, supplemental zinc improved weight gains and feed efficiency, and prevented dermatosis in pigs fed high-calcium diets.

High-calcium diets unsupplemented with zinc produced a significant drop in serum alkaline phosphatase.

The increased specific activity of certain tissues and the greater proportion of fecal zinc derived from endogenous sources suggested a higher rate of zinc metabolism in pigs receiving high-calcium diets unsupplemented with zinc.

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EFFECTIVENESS AND PRACTICABILITY OF SOME OILS, PENICILLIN, *n*-DECYL ALCOHOL, AND LECITHIN IN THE CONTROL OF ALFALFA BLOAT[1]

R. H. JOHNSON, L. R. BROWN, N. L. JACOBSON, AND P. G. HOMEYER

Iowa State College, Ames

NEW ZEALAND workers (Reid and Johns, 1957) have investigated the use of certain oils and paraffins as prophylactic and therapeutic measures against legume bloat. Penicillin has been used as an effective preventive by Barrentine *et al.* (1956) and Thomas (1956). Ross (1950) has described the use of various alcohols with eight or more carbon atoms per molecule as effective foam breakers in various fermentation and industrial processes. The surface tension of rumen fluid is depressed, both *in vivo* and *in vitro*, by lecithin (Nichols, 1957 and Nichols *et al.*, 1957) but the effect is of short duration. Johns (1956) noted that the antifoaming agents in general have an effect of short duration, but Reid (1957) found that emulsification slows the rate of loss of oils from the rumen. Previous work at this station (Johnson *et al.*, 1956) has shown that an emulsified oil markedly reduces bloat when administered in the drinking water or by other methods. The present study was designed to determine the effectiveness of several lipids, penicillin, and *n*-decyl alcohol in the prevention and treatment of bloat under various conditions and to investigate possible causes of the bloat syndrome. Preliminary reports have been presented (Johnson *et al.*, 1957; Brown *et al.*, 1957).

Experimental

In a series of trials during the spring and summer of 1957, 36 steers (average initial weight, 556 lb.) grazed alfalfa pasture of high bloat-producing potential. Considerable severe bloat resulted throughout most of the season although severity was noticeably less during the later trials. Animals grazed daily approximately from 6:30 to 10 a.m. and from 3:30 to 7 p.m. Immediately preceding grazing, all groups were offered equal amounts (on a per animal basis) of grain (concentrate) or grain mixed with preventive. In trial 1, one group received lard oil mixed in the drinking water; in all other trials the preventives were mixed with grain. Usually mixing was done at each feeding but in some cases several days' supply was prepared in advance and stored in a refrigerator until used. Due to its consistency, lecithin could not easily be mixed with grain; therefore, it was pre-mixed with oil and then incorporated into grain. During and for several hours after each grazing period the animals were under con-

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stant observation for bloat, which was evaluated subjectively on a scale from 0 to 5 (table 1). Preceding each trial individual animals were ranked by their cumulative bloat score over a period of several days. The three animals with the highest scores were randomly allotted to three groups; then the three animals with the next highest scores were similarly allotted and so on, so that on the basis of past performance the three groups used in each trial had approximately equal bloating potentials. Body weights were taken at the beginning and completion of each trial. Table 2 gives

TABLE 1. DESCRIPTION OF SCALE USED IN ASSIGNING BLOAT SCORES

Score	Description
0	No bloat—No distention in left paralumbar fossa
1	Slight—Slight distention in left paralumbar fossa; "puffy"
2	Mild—Marked distention in left paralumbar fossa; well rounded out between hip and rib on left side; little or no distention on right side
3	Moderate—Well rounded out on left side, drumlike; full on right side; restless
4	Severe—Both sides badly distended; left hip nearly hidden; skin tight; defecation; urination; incoordination; protruding anus; mild respiratory distress
5	Terminal—Extreme abdominal distention; severe respiratory distress; cyanosis; prostration; death unless treated

the experimental design. Choice white grease and a product composed of methyl esters of fatty acids [2] each were evaluated by addition to grain; at the relatively high level employed (17.5%), lack of palatability prevented adequate consumption of either mixture and use of these products was discontinued. These trials are not included in table 2 nor in any of the subsequent discussion.

In a concurrent series of trials, 22 dairy animals, varying in size, sex and breed, were divided into two comparable groups, confined to dry lot, and fed chopped alfalfa soilage *ad libitum* (except for a short period when birdsfoot trefoil was fed). Soilage was fed between 8 and 9 a.m.; that remaining at 6 p.m. was weighed back and removed. Observations of bloat were taken at least hourly while soilage was before the animals. The rating system employed is described in table 1 and the experimental design is shown in table 3. All animals were weighed at weekly intervals, and the treated and control groups were alternated every two weeks. Soybean oil was sprinkled over the soilage at the time of feeding and mixed in with a fork; *n*-decyl alcohol was administered orally in gelatin capsules.

When animals bloated severely, an attempt was made to release any free gas with a stomach tube. This usually was unsuccessful and an antifoaming agent was then administered by stomach tube or by hypodermic

[2] Lebcol T–40, supplied by Lyle Branchflower Co., Seattle, Washington.

TABLE 2. EXPERIMENTAL DESIGN, ALFALFA PASTURE TRAILS

Trial	Date	Grain[b]	Treatment[a] groups								
			1			2			3		
			Treatment	Level	Times daily	Treatment	Level	Times daily	Treatment	Level	Times daily
1	5/18–6/1	1.0	LO	0.25 lb.	2	...	Control	...	LO in water	2%	Continuous
2	6/2–6/15	1.5	LO	0.25 lb.	2	...	Control	...	LO in water	2%	Continuous
3	6/21–7/6	1.5	SBO	0.25 lb.	2	P	75 mg.	1	.....	Control	.....
4	7/7–7/20	1.5	SBO	0.25 lb.	2	P	125 mg.	1	.....	Control	.....
5	7/29–8/25	1.5	...	Control	...	L	0.19 lb.	2	L	0.19 lb.	2
						LO	0.06 lb.		SBO	0.06 lb.	
6	8/29–9/2	2.0	...	Control	...	L	0.27 lb.	2	L	0.27 lb.	2
						LO	0.09 lb.		SBO	0.09 lb.	
7	9/9–9/12	3.0	SBO	0.53 lb.	2	SBO	0.35 lb.	2	.....	Control	.....
8[c]	9/16–9/18	1.5	SBO	0.25 lb.	1	SBO	0.25 lb.	1	.....	Control	.....

[a] (LO) Lard oil—A water dispersible product derived from lard, supplied by Midwest Dried Milk Co., Dundee, Illinois.
(L) Lecithin—Alcolec, supplied by American Lecithin Co., Woodside, L. I., New York.
(P) Penicillin—Procaine penicillin supplied by Merck and Co., Inc., Rahway, New Jersey.
(SBO) Soybean oil—Crude soybean oil supplied by Spencer-Kellogg, Des Moines, Iowa.

[b] Total grain, or grain and treatment, lb. per animal per feeding.

[c] Animals received alfalfa soilage, *ad lib.*, in dry lot (all other trials on pasture).
(Group 1 received SBO sprinkled over soilage; Group 2 received SBO in grain.)

needle. In the pasture trials the agent employed was lard oil; in the soilage trials *n*-decyl alcohol was used.

Weather observations were made regularly. Rumen fluid samples were collected for analysis from animals with varying degrees of bloat and receiving the different treatments. Samples of forage were taken, both from the pasture plots and from the chopped soilage; these were analyzed for a number of components. Possible interrelationships among forage composition, rumen fluid characteristics, weather and incidence and severity of bloat were investigated. This phase of the study will be presented in a subsequent report.

TABLE 3. EXPERIMENTAL DESIGN, ALFALFA SOILAGE TRIALS

		Treatment groups			
		4		5	
Trial	Date	Treatment	Level[a]	Treatment	Level[a]
9	5/16–5/29	.... Control	.....	SBO	0.25 lb.
10	5/30–6/12	SBO	0.25 lb.	.... Control	.....
11	6/13–6/26[b]	.... Control	.....	SBO	0.25 lb.
12	6/27–7/10	SBO	0.25 lb.	.... Control	.....
13	7/11–7/24	.... Control	.....	SBO	0.25 lb.
14	8/3–8/16	DA	18 ml.	.... Control	.....
15	8/17–8/30	.... Control	.....	DA	18 ml.
16	8/31–9/13	SBO	0.25 lb.	.... Control	.....
17	9/14–9/16	.... Control	.....	SBO	0.25 lb.

[a] All soybean oil (SBO) was added at a rate of approximately 0.25 lb. per 1000 lb. body weight daily; *n*-decyl alcohol (DA), 18 ml. per 1000 lb.
[b] No alfalfa soilage available, birdsfoot trefoil soilage fed.

Results and Discussion

Results of the pasture trials are summarized in tables 4, 5, and 6, while table 7 presents those of the soilage trials. Analysis of variance of the pasture bloat observations (Snedecor, 1956) showed a statistically significant ($P<.05$ or $<.01$) decrease of average bloat severity and of average maximum bloat with all treatments employed except in trials 7 and 8. The non-significant differences in trials 7 and 8 probably resulted from a relatively low incidence and severity of bloat during these periods. This reasoning is supported by the very good control of bloat achieved in the soilage trials with soybean oil sprinkled over the soilage and in the earlier pasture trials where soybean oil in the grain was effective at much lower levels with a higher incidence and severity of bloat. The effect of penicillin, although statistically significant ($P<.01$) at 75 mg. per animal daily, was transitory; after 9 days the incidence and severity of bloat in the treated animals rose to approximately the same levels as the controls. Increasing the level of penicillin supplementation to 125 mg. per day in

trial 4 reduced bloat for 2 days, after which bloating was noticeably more severe and approached the level of the controls.

Analysis of variance of the weight gains indicated significant ($P<.05$) increases due to treatment in trials 1, 2, 3 and 4. In trials 5 and 6, lecithin plus soybean oil in the grain brought about a significant ($P<.05$)

TABLE 4. EFFECT OF TREATMENT UPON BLOAT SEVERITY, ALFALFA PASTURE TRIALS

Trial	Treatment[a]	Av./animal/day[b]	Av. max./ animal/day[c]
1	LO(g)	0.15	0.56
	LO(w)	0.17	0.76
	Control	0.39	1.34
2	LO(g)	0.13	0.47
	LO(w)	0.21	1.07
	Control	0.66	1.94
3	SBO(g)	0.09	0.46
	Penicillin(g)	0.20	0.87
	Control	0.46	1.49
4	SBO(g)	0.06	0.27
	Penicillin(g)	0.14	0.58
	Control	0.32	1.06
5	L & LO(g)	0.09	0.45
	L & SBO(g)	0.07	0.42
	Control	0.23	0.95
6	L & LO(g)	0.06	0.30
	L & SBO(g)	0.04	0.23
	Control	0.31	1.26
7	SBO (0.53 lb.) (g)	0.06	0.49
	SBO (0.35 lb.) (g)	0.17	0.85
	Control	0.29	1.15
8	SBO(s)	0.01	0.10
	SBO(g)	0.11	0.47
	Control	0.15	0.57

[a] LO=Lard oil, SBO=Soybean oil, L=Lecithin, g=mixed with grain, w=mixed with water, s=sprinkled over soilage (see table 2 for levels).

[b] Average of all observations taken periodically throughout day (see table 1 for explanation of bloat ratings).

[c] Average of maximum values for each animal.

increase in gains but there were no real differences between the control animals and those receiving lecithin and lard oil. Table 5 shows that increased gains due to treatment ranged from 0.07 lb. to 0.75 lb. per day.

Considerable difficulty was experienced in inducing some animals to consume the grain treated with the lard oil and lecithin mixture (trials 5 and 6), perhaps because the mixture became quite lumpy and hard, even during a short storage period. Soybean oil was the most palatable

oil employed and nearly all animals relished it. Lard oil (trials 1 and 2) appeared less palatable than soybean oil possibly because it was the first oil fed in the grain. There was a marked improvement in consumption as lard oil feeding continued. Previous experience had shown the oil to be very palatable when mixed with water (Johnson *et al.*, 1956).

In several trials (particularly 1, 2, 5 and 6) treatment effect was more pronounced when adjustments were made for animals in the treated groups which failed to consume the treated grain. Values in table 4 include all animals in each treatment group, even though some of the animals refused to eat treated grain (occasionally or frequently). Data in table 6,

TABLE 5. EFFECT OF TREATMENT UPON WEIGHT GAINS, ALFALFA PASTURE TRIALS

Treatment[a]	Average daily gain lb.	Increase over control lb.
LO(g)	1.84	0.62
LO(w)	1.97	0.75
Control	1.22	
SBO(g)	1.54	0.72
Penicillin(g)	1.26	0.44
Control	0.82	
L & LO(g)	1.56	0.07
L & SBO(g)	1.73	0.24
Control	1.49	

[a] See table 2 for explanation of symbols.

however, show the difference in bloating behavior during trials 1, 2, 5, and 6 among the animals in the treated groups which consumed the treated grain at certain times but not at others. The tendency not to eat the treated grain was less noticeable during the remaining periods (although one animal consistently refused to eat any grain and oil) and these trials are not represented in table 6. Data in table 6 indicate that there apparently was a carry-over effect of treatment from morning to evening (animals treated in the morning but not the same evening did not bloat as much that evening as did the controls). The carry-over of treatment effect from evening to the next morning was somewhat less, probably because of the longer time period. A prolonged effect of oil when fed at a high level has been demonstrated by previous work (Brown *et al.*, 1958) in which animals receiving one pound of lard oil experienced no bloat for 12 to 14 hours during which time several control animals died or required drastic treatment.

At least part of the lower efficiency of lard oil in the drinking water when compared to administration of the same type of oil in grain probably was due to decreased consumption of treated water during and

TABLE 6. EFFECT OF REGULARITY OF TREATMENT UPON BLOAT SEVERITY, ALFALFA PASTURE TRIALS

Regularity of treatment	Average bloat severity			
	Daily average[a]	Daily maximum[b]	AM maximum[b]	PM maximum[b]
00[c]	0.57	1.71	1.28	1.33
01[d]	0.25	0.90	0.90	0.48
10[e]	0.15	0.84	0.23	0.57
11[f]	0.05	0.29	0.13	0.17

[a] Average of all observations taken periodically throughout day (see table 1 for explanation of bloat ratings).
[b] Average of maximum values for each animal.
[c] Consumed no treated grain.
[d] Consumed no treated grain in a.m., consumed treated grain in p.m.
[e] Consumed no treated grain in p.m., consumed treated grain in a.m.
[f] Consumed treated grain, a.m. and p.m.

shortly after rainy periods. Cool weather during trials 1 and 2 also reduced water (and oil) intake to some extent.

Crude soybean oil sprinkled over the 24-hour allotment of alfalfa soilage at the daily rate of approximately 0.25 lb. per 1000 lb. of body weight effectively controlled bloat. As might be expected, the effect was most striking during the period of severe bloat, and analysis of variance (Snedecor, 1956) showed a significant effect ($P<.01$). The effect of soybean oil approached significance at $P=.05$ when the incidence and severity of bloat were moderate. Examination of table 7 reveals that there was some reduction of bloat where soybean oil was used during periods of mild bloat but the reduction was not statistically significant at $P=.05$. Weight gains were extremely variable and no conclusions could be reached concerning the effect of treatment.

TABLE 7. GENERAL EFFECT OF TREATMENT UPON BLOAT SEVERITY AND WEIGHT GAINS, ALFALFA SOILAGE TRIALS

Bloat	Trial (from table 3)	Treatment[a]	Bloat severity			Wt. gains, lb./day	
			Scale	Treated	Control	Treated	Control
Mild	13	SBO	Average	0.02	0.06	1.43	2.35
			Av. max.	0.14	0.28		
	14, 15	DA	Average	0.06	0.07	0.79	0.60
			Av. max.	0.18	0.21		
Moderate	9, 10, 12, 16	SBO	Average	0.04	0.18	2.28	1.91
			Av. max.	0.13	0.64		
Severe	17	SBO	Average	0.06	0.72	3.42	−5.47
			Av. max.	0.24	2.30		

[a] SBO=Soybean oil, DA=*n*-decyl alcohol.

The *n*-decyl alcohol apparently had very little prophylactic effect; however, it was used only during periods of mild bloat. As in the case of soybean oil, n-decyl alcohol may be more effective when bloat is more severe. During the first 1 to 2 hours after administration of the alcohol there was less bloat among the treated animals; thereafter, the effect of treatment was less apparent. The effect seems to be short lived, probably due to rapid alteration in or removal from the rumen.

During trial 11 alfalfa soilage was not available and birdsfoot trefoil soilage was fed. This soilage was of good quality and was cut at about the one-fourth bloom stage. Considerable difficulty was experienced initially in getting animals to eat the soilage but after the third day consumption improved markedly and by the end of the first week intake of trefoil soilage approximately equalled that of alfalfa soilage. No cases of bloat were observed during the time the animals were fed the trefoil; neither were there any other adverse effects, such as HCN toxicity.

Apart from the reluctance of some of the animals to consume the lard oil or lard oil-lecithin mixtures there were no other indications that feed intake, growth, and general well-being were in any way adversely affected by any of the materials employed in these studies. On the contrary, weight gains were increased markedly in many cases, a fact which makes it possible to charge part of the cost of the preventive to weight gains and to make the actual prevention of bloat more feasible from an economic standpoint.

In severe cases of bloat the oral administration of 150–250 ml. of lard oil was followed soon by the release of large quantities of gas from the rumen via stomach tube and/or eructation. Recovery was usually complete (table 8) within 30 to 40 minutes and animals so treated did not bloat before the next grazing period, even when they returned to pasture and resumed eating. In the soilage trials the therapeutic agent used was *n*-decyl alcohol in approximately 25 ml. doses. The alcohol caused a prompt reduction in bloat, but the effect lasted for only about 2 hours.

Summary

In one series of trials, 36 steers on alfalfa pasture received various prophylactic agents in dry feed before grazing twice daily or in water. Soybean oil, lard oil, and lecithin mixed with soybean oil greatly reduced bloat for several hours when fed at the rate of 0.25 lb., or more, per animal in the grain at each feeding or at the rate of 2% in the drinking water. Feeding oil or oil-lecithin mixtures increased weight gains appreciably, ranging from 0.07 to 0.75 lb. per animal per day for the various oil treatments. Penicillin (75 mg. per animal daily) reduced bloat for nine days, but subsequently its effectiveness diminished rapidly. Increasing the penicillin to 125 mg. reduced bloat for a period of two days, after which bloat

incidence increased sharply. Animals receiving penicillin gained 0.44 lb. per day more than controls.

In another series of trials, 22 dairy animals received chopped alfalfa soilage. Crude soybean oil sprinkled on the soilage at a level of approximately 0.25 lb. per 1000 lb. body weight of animal per day effectively controlled

TABLE 8. RESPONSE OF BLOATED ANIMALS TO TREATMENT WITH LARD OIL OR *n*-DECYL ALCOHOL

Bloat severity[a]	Level (ml. agent per treatment)	No. animals treated		Response of animals		
		1956[b]	1957[c]	No. recovered	No. requiring other treatment	No. died
			Lard oil			
3	100	2	1	3		
	150		6	6		
	200		10	10		
	250		2	2		
4	100	8	6	13	1	
	150	1	10	11		
	200		4	4		
	250					
	300		1	1		
5	100	1	5	4		2
	150		5	4		1
	200		1		1	
	250		1	1		
	300					
	350		1	1		
			n-Decyl alcohol			
4	25		2	2		
	100		3	3		

[a] See table 1 for description of bloat severity ratings.
[b] All treatment was by intraruminal injection (1956 data from Brown *et al.*, 1958).
[c] All treatment was by stomach tube.

bloat. The effect of *n*-decyl alcohol was of too short duration for satisfactory prophylaxis.

Lard oil and *n*-decyl alcohol administered intraruminally were successful in relieving very severe cases of bloat, apparently by breaking the foam and releasing large quantities of free gas in a short time. The lard oil was the more satisfactory because of ease of administration and promptness and duration of effect.

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NEWS AND NOTES

The 50th Anniversary meeting of the American Society of Animal Production will be held at the Hotel Sherman, Chicago, Friday and Saturday, November 28–29, 1958. There will be sectional meetings for the presentation of research papers from 9:30 a.m. to 11:00 a.m. and from 1:00 p.m. to 4:00 p.m. on Friday and a general session from 11:00 a.m. to 12:00. A dinner will be held at 6:00 p.m., Friday evening which will honor past presidents and charter members. The dinner, as a special event, will take the place of the usual Saturday noon luncheon. The annual business meeting will be held after the dinner. Sectional meetings will be held on Saturday from 9:00 a.m. to 12:00, 1:00 p.m. to 4:00 p.m. and 7:30 p.m. to 9:30 p.m.

In recognition of the 50th Anniversary of the American Society of Animal Production, the November issue of the Journal of Animal Science will feature a series of review papers each covering 50 years of progress in a particular phase of animal production or related area of research and teaching. These invitational review papers are being assembled and arranged by J. K. Loosli, former editor of the Journal. In addition to these reviews, the issue will contain abstracts of papers submitted for presentation at the 50th annual meeting and the usual indices for the current volume.

At the annual meeting of the North Atlantic section of the American Society of Animal Production, June 27, the following officers were elected for the year 1959–60: President, Willard W. Green, University of Maryland; Vice President, W. A. Cowan, University of Connecticut; Secretary-Treasurer, George W. Vander Noot, Rutgers, The State University.

A specialized service to State agricultural experiment stations providing for consultation and comprehensive review of research programs, including those in Animal Science, is provided by the State Experiment Stations Division, ARS, USDA, in cooperation with the Station Directors. The Directors are familiar with details of the service, but this statement may be of interest to animal scientists and department heads.

Such reviews provide opportunity for discussion of problems which affect the agriculture of the State, and of current and proposed research directed toward their abatement or solution. Each review encompasses several days of consultations between visiting panels of specialists and the Director and staff. The panels include two or more consultants recruited from other experiment stations, universities, or industry, and one or two representatives of SESD. The review may deal with the program of one department or problem areas that involve two or more departments. They permit a much more comprehensive activity than is possible during the annual reviews by the staff of the State Experiment Stations Division. The comprehensive review stems from the idea that workers wish to review their own research from time to time with the assistance of scientists from other institutions who have different viewpoints and experiences and special competence in certain lines of research.

The reviews are undertaken by SESD only upon request of the Station Director with no thought of attempting to exercise any measure of direction or control. The chief benefits are likely to result from exchange of ideas and stimulation to new thought rather than from specific suggestions concerning details of research projects.

A total of 20 such reviews were conducted during the past year, and a comparable number are being planned for next fall and winter.

Enos J. Perry, Extension Specialist in Dairy Husbandry at Rutgers from 1924 to 1956, has recently returned from Lebanon where he served for 16 months as animal husbandry advisor with the International Cooperation Administration's mutual security program. He helped to set up a program designed to lower the cost of milk production and increase the nation's supply of milk. Imported cattle and their progeny from

Holland are presently providing from 30 to 50 percent more milk than similar milk cattle of Lebanon. An artificial insemination program introduced in the country upon his recommendations and described in the Arabic language for use by cattle raisers in Lebanon has resulted in improving breeds throughout the nation.

Paul B. Pearson has accepted a position with the Program in Science and Engineering of the Ford Foundation. Dr. Pearson has been with the U. S. Atomic Energy Commission since 1949 as Chief of the Biology Branch. He also holds a professorship in the Biology Department at the Johns Hopkins University. In his new position with the Ford Foundation he will be responsible for the program in the natural sciences.

L. J. Horlacher, on leave from University of Kentucky, has been serving for 20 months in India as Administrator Advisor for the University of Tennessee—India Agricultural Program. He is now in Guatemala as Group Leader for the University of Kentucky—Guatemala Agricultural Program.

M. A. Schooley, D.V.M., has been appointed to the new position of Director of Animal Health and Nutrition Applied Research, Armour and Company. Dr. Schooley joined Armour in 1951, and has been associated with veterinary pharmaceutical research.

Thomas M. Means who received his Ph.D. degree from Purdue University in 1956 has been named assistant head in charge of animal nutrition research at the Lilly Agricultural Research Center, Eli Lilly and Company.

J. C. Miller, Dean of Agriculture, Texas A and M College System, since October 1956, resigned effective June 30, 1958, to accept appointment at Oregon State College as head of their new Department of Dairy and Animal Husbandry.

W. C. Weir, Associate Professor of Animal Husbandry, became Dean of Students at the University of California, Davis campus, on July 1, 1958. He will continue teaching and research in the Animal Husbandry Department on a half-time basis.

Fred F. McKenzie, Professor of Animal Husbandry, returned to Oregon State College in July 1958, following a two-year leave of absence. During this period he served with the ICA program of the University of Nebraska, working at Atatürk and Ankara University in Turkey.

O. Burr Ross has been named Head of the Department of Animal Science at the University of Illinois. Previous to this appointment, he was manager of the Gooch Feed Mill, Saline, Kansas, and was formerly on the Animal Husbandry staff at Oklahoma State University.

Earl L. Lasley joined the research staff of the William H. Miner Agricultural Research Institute, Chazy, New York, as Senior Scientist, effective August 1, 1958. He was formerly Associate Professor of Animal Science at the University of Illinois.

Luther A. Weaver, Professor of Animal Husbandry, University of Missouri, and head of that department for many years, retired August 1957.

William H. Pfander, Professor of Animal Husbandry at the University of Missouri, has been awarded a senior postdoctoral fellowship by the National Science Foundation for the coming academic year. He will study acetate metabolism, one of the fundamental problems in ruminant nutrition at the Commonwealth Scientific and Industrial Research Organization, Sheep Biology Laboratory at Prospect, near Sydney, Australia.

On May 26, 1958, W. D. Salmon, former Head of the Department of Animal Husbandry and Nutrition at the Alabama Polytechnic Institute was awarded an honorary Doctor's degree by the University of Kentucky for his many contributions

in the field of human and animal nutrition. Dr. Salmon served 36 years on the staff of API and was Department Head from 1950 to 1957.

C. V. Ross joined the Animal Husbandry staff at the University of Missouri early this year with the rank of Assistant Professor. He will be engaged in teaching and research in sheep production. Dr. Ross was formerly employed by Swift and Company in lamb feeding work.

Floyd W. Bell, Professor of Animal Husbandry at Kansas State College, retired June 30, 1958.

Robert W. Morrison of Toronto, Canada, has been on a three months appointment as Dairy Consultant at FAO headquarters in Rome.

A. M. Mullins, former instructor in Animal Husbandry at the University of Missouri, has joined the Animal Industry staff of the Louisiana Agricultural Experiment Station.

John F. Lasley, Professor of Animal Husbandry, University of Missouri, began a year of sabbatical leave February 1, 1958.

Philip A. Anderson, Associate Professor of Animal Husbandry at the University of Minnesota, retired in June 1958 after 43 years. He made important contributions through teaching and research in sheep production and meats. R. M. Jordan and W. J. Aunan have assumed Prof. Anderson's former duties.

Bill Neil Day, has been appointed Assistant Professor of Animal Husbandry at the University of Missouri effective September 1, 1958. Day's major field of study has been animal breeding. At the University of Missouri he will be teaching and doing swine research work.

A. G. Van Horn, superintendent of the Dairy Experiment Station, Lewisburg, Tennessee, operated by the Tennessee Agricultural Experiment Station and USDA, retired June 30, 1958.

G. H. Schmit is a new member of the Animal Husbandry Department at Cornell University with the rank of Assistant Professor.

D. C. Clanton and L. J. Sumption have been appointed to the Animal Husbandry staff at the University of Nebraska, each with rank of Assistant Professor.

E. V. Dillard, Assistant Professor in the Animal Industry Department at North Carolina State College, is on an assignment in Peru, South America, under the ICA contract of N.C.S.C.

Joseph V. Scaletti who received his Ph.D. degree in Bacteriology at Cornell University in 1956, has joined the University of Minnesota Department of Animal Husbandry as Assistant Professor.

Donald H. Kropf who has been serving with the U. S. Army Quarter Master Institute since completion of his Ph.D. degree in Animal Husbandry at the University of Wisconsin, has joined the staff of the Animal Husbandry Department, Clemson College, Clemson, South Carolina.

Harold H. Pierce has been appointed Superintendent of the Clemson College Coast Experiment Station, Summerville, South Carolina. He received his B.S. and M.S. degrees from the University of Georgia, and Ph.D. degree from the University of Illinois.

The Guinea Pig in Research by MARY ELIZABETH REID; 87 pp. $2.00; publication number 557, Human Factors Research Bureau, Inc., National Press Building, Washington 4, D. C., 1958.

This pamphlet covers in a brief manner such topics as genetics of the guinea pig, vital data, some aspects of its physiology, and nutrition research; the greater proportion of the last section being devoted to vitamins. Brief mention is made of use of the guinea pig in the study of infectious diseases, sensitization, pharmacological research and effects of radiation. There as 7 halftone plates, 12 tables and a bibliography of 227 references.

The first volume of a three-volume work on animal breeding—*Handbuch der Tierzuchtung*—was published in Germany in May 1958. The "Handbuch" is being edited by Ivar Johnssen, John Hammond and F. Haring. This first volume, "Biological Basis of Animal Production," contains a chapter on animal ecology (Tierökologie) by Ralph W. Phillips. Other chapters deal with origin and domestication of farm animals; reproduction disturbances; artificial insemination; growth; secretion and composition of milk; wool and wool quality; egg production and quality; poultry for meat production; recording of performance in farm animals; and the principles of judging farm animals; each chapter has been prepared by a specialist in that field. Published by Paul Parey, Hamburg and Berlin, the first volume is priced at 98 German marks, and the other volumes at 88 marks each. The second volume will deal with the genetics of farm animals and the third with breeds of farm animals.

Mirror to Physiology; A Self Survey of Physiological Science by R. W. GERARD; 372 pages; $5.00; American Physiological Society, Washington, D. C., 1958.

A pertinent summary of the activities, training, experience, salaries, and other vital information from replies to questionnaires sent to American and Canadian physiologists, both animal and plant, has been carefully prepared and is presented with the use of numerous tables, charts and graphs. A large percent of all physiologists responded to the questionnaires and results obtained seem to reflect quite adequately the present status and relevant historical conditions of direct concern to the physiologists. Although many of the animal physiologists responding to this questionnaire were not in the area of animal husbandry, it is felt that many of the results apply directly to the physiologist in this area and to many of the other members of animal science departments.—*M. J. B.*

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts." *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. W. D. Gallup, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma, or to the publishers: Boyd Printing Company, 372–378 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. H. H. Stonaker, Department of Animal Husbandry, Colorado State University, Fort Collins, Colo., or to the publishers, Boyd Printing Company, 372–378 Broadway, Albany 7, New York.